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Mercury Methylation and Bioaccumulation in Sinclair Inlet, Kitsap County, Washington

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By A.J. Paulson, M.C. Marvin-DiPasquale, P.W. Moran, J.F. Stewart, J.F. DeWild, J. Toft, J.L. Agee, E. Kakouros, L.H. Kieu, B. Carter, R.W. Sheibley, J. Cordell, and D.P. Krabbenhoft

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RYAN K. ZINKE, Secretary

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James F. Reilly II, Director

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Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
Length		
foot (ft)	0.3048	meter (m)
Volume		
gallon (gal)	3.785	liter (L)

International System of Units to U.S. customary units

Multiply	By	To obtain
Length		
centimeter (cm)	0.3937	inch (in)
micrometer (μm)	0.003937	inch (in)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
Area		
square kilometer (km^2)	0.3861	square mile (mi^2)
square centimeter (cm^2)	0.001076	square foot (ft^2)
Volume		
kilogram per liter (kg/L)	8.3454	pound per gallon (lb/gal)
milliliter (mL)	0.0338	ounce, fluid (fl. oz)
liter (L)	0.2642	gallon (gal)
Mass		
milligram (mg)	3.527	ounce (oz)
Flow rate		
meter per second (m/s)	3.2808	foot per second (f/s)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as:

$$°F = (1.8 \times °C) + 32.$$

Datums

Vertical coordinate information is referenced to National Geodetic Vertical Datum of 1929 (NGVD 29).

Horizontal coordinate information is referenced to North American Datum of 1983 (NAD 83).

Altitude, as used in this report, refers to distance above the vertical datum.

Supplemental Information

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L), micrograms per liter (μg/L), or nanograms per liter (ng/L).

Concentrations of chemical constituents of solids are given in either percentage of dry weight, milligrams per kilogram (mg/kg) or nanograms per milligram (ng/mg), which are equivalent. Nanogram per gram (ng/g) approximately equals parts per billion. Nanogram per square meter per day ($[ng/m^2]/d$) is concentration produced per area per day.

Abbreviations

AIC	Akaike Information Criterion
ANOVA	analyses of variance
ASSR	ArcSine square root (data transformation)
AVS	acid-volatile sulfur (sediment)
BD	bulk density
BNC	Bremerton naval complex
BI	Budd Inlet
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CTD	conductivity, temperature, and depth
CVAFS	cold-vapor atomic fluorescence spectrometry
CZ	convergence zone
DOC	dissolved organic carbon
E_h	reduction-oxidation potential
ENVVEST	ENVironmental inVESTment
Fe(II)	ferrous iron
$Fe(II)_{AE}$	acid-extractable ferrous iron (sediment)
$Fe(III)_a$	amorphous (poorly crystalline) ferric iron (sediment)
$Fe(III)_c$	crystalline ferric iron (sediment)
Fe_T	total iron (sediment)
FMHg	filtered methylmercury
FP	fluorocarbon polymer
FTHg	filtered total mercury
GSI	greater Sinclair Inlet
H_2S	hydrogen sulfide
HCl	hydrochloric acid
HDPE	high-density polyethylene
Hg^0	elemental mercury

Abbreviations

*hgc*AB   mercury (II)-methylation gene cluster

Hg(II)   mercury(II), an oxidative state commonly found in inorganic salts of mercury

HH   Holmes Harbor

k_{meth} methylmercury production rate constant

KOH   potassium hydroxide

KWRS   Kruskal-Wallis Rank Sum

LB   Liberty Bay

ln   Natural log

LOI   loss of ignition

MHg   methylmercury

Mn   manganese

MPP   methylmercury production potential

NBK   Naval Base Kitsap Bremerton

N_2 nitrogen gas

NRP   National Research Program (USGS)

OU B   Operable Unit B (includes "OU B Marine" and "OU B Terrestrial")

PCB   polychlorinated biphenyl

PETG   polyethylene terephthalate glycol

PFA   perfluoroalkoxy copolymer

PMHg   particulate methylmercury

PSNS   Puget Sound Naval Shipyard

PTFE   polytetrafluoroethylene

PTHg   particulate total mercury

QFF   quartz fiber filter

r   Pearson correlation coefficient

R^2 coefficient of determination

redox   reduction-oxidation

rpm   revolution per minute

RPD   relative percent difference

SI   Sinclair Inlet

SI-IN   Sinclair Inlet-Inner

SI-OUT   Sinclair Inlet-Outer

SI-PO   Sinclair Inlet-Port Orchard

SMHg   sediment methylmercury

SRHg   sediment reactive inorganic mercury

STHg   sediment total mercury

Abbreviations

$\delta^{13}C$	stable isotope of carbon
$\delta^{15}N$	stable isotope of nitrogen
THg	total mercury
TRS	total reduced sulfur (sediment)
USGS	U.S. Geological Survey
WAWSC	Washington Water Science Center (USGS)
WMRL	Wisconsin Mercury Research laboratory (USGS)
WRS	Wilcoxon Rank Sum
YSI	Yellow Springs Instruments Company, Incorporated

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Abstract

The U.S. Geological Survey evaluated the transformation of mercury to bioavailable methylmercury in Sinclair Inlet, Kitsap County, Washington, and assessed the effect of the transformation processes on the mercury burden in marine organisms and sediment. In August 2008, samples of sediment, water, and biota from six sites in Sinclair Inlet and three bays representative of Puget Sound embayments were collected. The extensive sediment sampling included analysis of methylmercury in sediment and porewater, estimates of methylation production potential, and analyses of ancillary constituents associated with organic carbon and reduction-oxidation (redox) conditions to assist in interpreting the mercury results. Analyses of methylmercury in water overlying incubated cores provided an estimate of the release of methylmercury to the water column. Collection of samples for mercury species in the aqueous, particulate (suspended solids), and biological phases, and for ancillary carbon and nitrogen constituents in surface water, continued, on about a monthly schedule, at four stations through August 2009. In February, June, and August 2009, seasonal sediment samples were collected at 20 stations distributed between greater Sinclair Inlet and Operable Unit B Marine of the Bremerton naval complex, Bremerton, Washington, to examine geographical and seasonal patterns of mercury biogeochemistry of sediment in Sinclair Inlet. At six of these seasonal sediment stations, porewater was collected and triplicate core incubation experiments were done.

Median sediment-methylmercury concentrations were not statistically different between the representative bays and Sinclair Inlet. The percentage of sediment methylmercury (relative to total mercury) was actually lower in the Sinclair Inlet sites compared with the representative bays, reflecting the higher sediment total mercury concentration for the Sinclair Inlet stations compared with the representative bays. Likewise, median sediment methylmercury concentrations were not statistically different between the greater Sinclair Inlet stations and the Bremerton naval complex stations; whereas the percentage of sediment methylmercury to total mercury was lower in the Bremerton naval complex due to higher sediment total mercury concentrations than the greater Sinclair Inlet stations. The biogeochemical characteristics of each station, measured by redox, organic carbon, and the seasonal availability of nutrients controlled methylmercury biogeochemistry. Mercury methylation production potential was a function of temperature, concentration of total mercury in sediment, and the percentage of ferrous iron (relative to total measured iron) across all sites. Methylmercury porewater concentrations were best described by using concentrations of dissolved organic carbon and reduction-oxidation conditions. Likewise, the variable fluxes of methylmercury from incubated cores were best described using dissolved organic carbon and reduction-oxidation conditions.

Sinclair Inlet exhibited the classic Puget Sound biological cycle, with spring and autumn phytoplankton blooms resulting in depletion of nitrate, orthophosphate, and silicate in the surface water. Although variable in timing between 2008 and 2009, a strong corresponding seasonal trend of increased availability, incorporation, and bioaccumulation of methylmercury into the food web of Sinclair Inlet occurred during the early spring and summer growing season.

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[1]U.S. Geological Survey, retired.

[2]U.S. Geological Survey.

[3]University of Washington, School of Fisheries and Aquatic Sciences, Wetland Ecosystem Team.

[4]Washington State Department of Health, Office of Drinking Water, Northwest Regional Office.

I.   Introduction and Methods

By A.J. Paulson, M.C. Marvin-DiPasquale, and
P.W. Moran

As early as the 1980s, the sediment in Sinclair Inlet was identified as having increased concentrations of a number of elements and organic compounds (Malins and others, 1982). A remedial investigation of the marine waters off the Bremerton naval complex (BNC), Bremerton, Washington, was completed in 1996 (U.S. Navy, 2002), and the Record of Decision (U.S. Environmental Protection Agency, 2000) was issued as final in 2002. The remediation option included isolating a considerable volume of contaminated sediment from interactions with the benthic food web by capping and disposing of dredge spoils in a covered, confined aquatic disposal pit in 2001. The primary objective of the marine sediment cleanup was to address the potential risk to humans, particularly those engaged in a subsistence lifestyle, from consumption of bottom-dwelling fish known to have polychlorinated biphenyls (PCBs) in their tissues (U.S. Navy, 2002). Three pathways were identified as having the capability to transport chemicals from the terrestrial landscape of the BNC to the marine environment, and thus as having the potential to re-contaminate the recently remediated marine sediment. The pathways included discharge directly from dry docks, discharge of groundwater directly to marine waters, and discharge of stormwater from facilities handling surface-water runoff.

As lead agency for environmental cleanup of the BNC, the U.S. Navy completed the second 5-year review of the remedial actions of the marine sediment in the boundary of the BNC (U.S. Navy, 2007); pursuant to Section 121(c) of the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA; Public Law 107-377) and the National Oil and Hazardous Substances Pollution Contingency Plan (40 Code of Federal Regulations Part 300). One of the issues in the second 5-year review highlighted by the cooperator, Naval Facilities Engineering Command was that, "There is insufficient information to determine whether the remedial action taken at OU [Operable Unit] B Marine with respect to mercury in sediment is protective of ingestion of rockfish by subsistence finfishers" (U.S Navy, 2007, p. 5).

Recommendations and follow-up actions in the 5-year review were:

- Revisit Remedial Investigation/Feasibility Study (RI/FS) ground-water-to-surface-water transport evaluations in light of total mercury concentrations in two long-term monitoring wells,

- Perform trend analyses and assess functionality and protectiveness of remedy for marine sediment, and

- Collect additional information necessary to perform a risk evaluation and reach conclusions regarding the protectiveness of the remedy (U.S. Navy, 2002) with respect to total mercury concentrations in Sinclair Inlet sediment and fish tissue.

Purpose and Scope

Since 2007, the U.S. Geological Survey (USGS) and the U.S. Navy have started several multi-year studies. The objectives were to (1) estimate the magnitudes of the different predominant sources of total mercury to Sinclair Inlet, including those from the BNC, (2) evaluate the transformation of mercury to a bioavailable form in Sinclair Inlet, and (3) assess the effect of the sources and transformation processes on the mercury burden in marine organisms and sediment. The initial Watershed Sources Project, which focused on the first objective, synthesized existing data of total mercury (THg) in sediment, water, and biota of Sinclair Inlet (Paulson and others, 2010) and assessed sources of filtered and particulate (suspended solids) mercury to Sinclair Inlet[5] (Paulson and others, 2012, 2013).

This report documents the Methylation and Bioaccumulation Project, which focused on the second and third objectives. The specific tasks completed to achieve these objectives were:

- Task 1—Assess the seasonal probability that sedimentary Hg throughout Sinclair Inlet may be methylated.

- Task 2—Confirm Task 1 by intensively examining the porewaters of Sinclair Inlet sediments and the releases of total mercury and methylmercury from Sinclair Inlet sediments using incubated sediment-core experiments.

- Task 3—Determine the spatial and temporal variability of methylmercury concentrations in zooplankton and, as feasible, phytoplankton in Sinclair Inlet relative to the spatial and temporal variability of dissolved and particulate concentrations of total mercury and methylmercury in the water.

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[5] Several types of mercury measurements were collected during this study. Various forms of mercury herein are abbreviated as total mercury (THg), methylmercury (MHg), particulate (typically collected onto a filter) total mercury (PTHg), particulate methylmercury (PMHg), filtered total mercury (FTHg), and filtered methylmercury (FMHg).

Site Description

Sinclair Inlet

Sinclair Inlet (SI), a shallow embayment (maximum depth of 20 meters [m]) is on the west side of the Puget Sound lowlands, (fig. 1). The Puget Sound lowland is a long, northward-trending structural depression between the Cascade Mountains on the east and the Olympic Mountains on the west. Most of the Puget Sound lowland physiographic province is mantled with thick glacial and postglacial deposits.

The Sinclair Inlet-Dyes Inlet system is hydrologically complex not only because of the geometry of the Sinclair Inlet-Dyes Inlet connection, but Bainbridge Island blocks the connection between the Dyes Inlet-Sinclair Inlet system and central Puget Sound (fig. 1). The Dyes Inlet-Sinclair Inlet system is connected to central Puget Sound through Port Orchard Passage on the north side of Bainbridge Island and through Rich Passage on the south side of Bainbridge Island (fig. 1). The maximum depth of Rich Passage is 20 m and the maximum depth of Port Orchard Passage is 6 m. The shallowness of these passages results in extensive vertical mixing of the incoming tidal water. Tides in Puget Sound are mixed diurnally and have a maximum tidal range of about 5 m relative to a maximum depth of about 20 m for Sinclair Inlet. The relative proportion of tidal volumes through Port Orchard Passage and Rich Passage is unknown. Because the tidal prism volume of Dyes Inlet is about three times that of Sinclair Inlet, tidal currents in Port Washington Narrows (fig. 2), which connects Dyes Inlet to Sinclair Inlet, often lag those of Sinclair Inlet (Wang and Richter, 1999). Further, the convergence of strong tidal currents south of Port Washington Narrows, which drains Dyes Inlet, and east of Sinclair Inlet proper where strong tides and extensive mixing has been shown (Wang and Richter, 1999), is defined here as the convergence zone (CZ).

Bremerton Naval Complex

The Bremerton naval complex (approximately about 2 square kilometers [km^2]) is located on the north shore of Sinclair Inlet in Bremerton, Washington (fig. 3) and contains Puget Sound Naval Shipyard (PSNS) and the Naval Base Kitsap Bremerton (NBK Bremerton).

The primary role of PSNS (1.5 km^2) is to provide overhaul, maintenance, conversion, refueling, defueling, and repair services to the naval fleet. The PSNS, which can dry dock and maintain all classes of Navy vessels, is the Nation's sole nuclear submarine and ship recycling facility. The PSNS occupies the eastern part of the complex and has six dry docks, eight piers and moorings, and numerous shops to support its industrial operations. This fenced high-security area hosts many tenant commands.

The primary role of NBK Bremerton, which occupies the western part of the naval complex, is to serve as a deep-draft homeport for aircraft carriers and supply ships. The facility is a fenced and secure area that extends into Sinclair Inlet. Facilities on the NBK Bremerton property (0.4 km^2) include six piers and moorings, a steam plant, parking lots, housing, stores, recreation areas, and eateries. NBK Bremerton is responsible for providing long-term care of inactive naval vessels. For the purposes of environmental remediation, the BNC was divided into Operable Units (OU) OU A, OU B, OU C, OU D, and OU NSC. Subsequently, OU B was further divided into OU B Terrestrial and OU B Marine. Of the OUs, only data previously collected from OU B Marine (fig. 3) are addressed in this report. For the purposes of this report, the greater Sinclair Inlet (GSI) is defined as the area outside of OU B Marine of the BNC and includes the station in the CZ.

Representative Bays

The three representative bays (fig. 1) selected for this study are similar to Sinclair Inlet in size, depth, and geometry. Holmes Harbor is an embayment adjacent to rural Whidbey Island, whereas Budd Inlet (BI) is adjacent to Olympia, the capital city of Washington. Similar to Sinclair Inlet, Liberty Bay is connected to Port Orchard Passage and is adjacent to the suburban town of Poulsbo.

History of Remediation and Environmental Investigations Related to Mercury

A synthesis of data related to THg concentrations in sediment throughout Puget Sound indicated that THg concentrations in sediment in OU B Marine were higher than other urban areas of Puget Sound (Evans-Hamilton, Inc., and D.R. Systems, Inc., 1987). In 1989, the State of Washington Puget Sound Ambient Monitoring Program began monitoring the marine waters and sediment of Puget Sound.

Figure 1.   Sinclair Inlet and locations of representative bays in Holmes Harbor (HH), Budd Inlet (BI), Liberty Bay (LB), Puget Sound, Washington, August 2008.
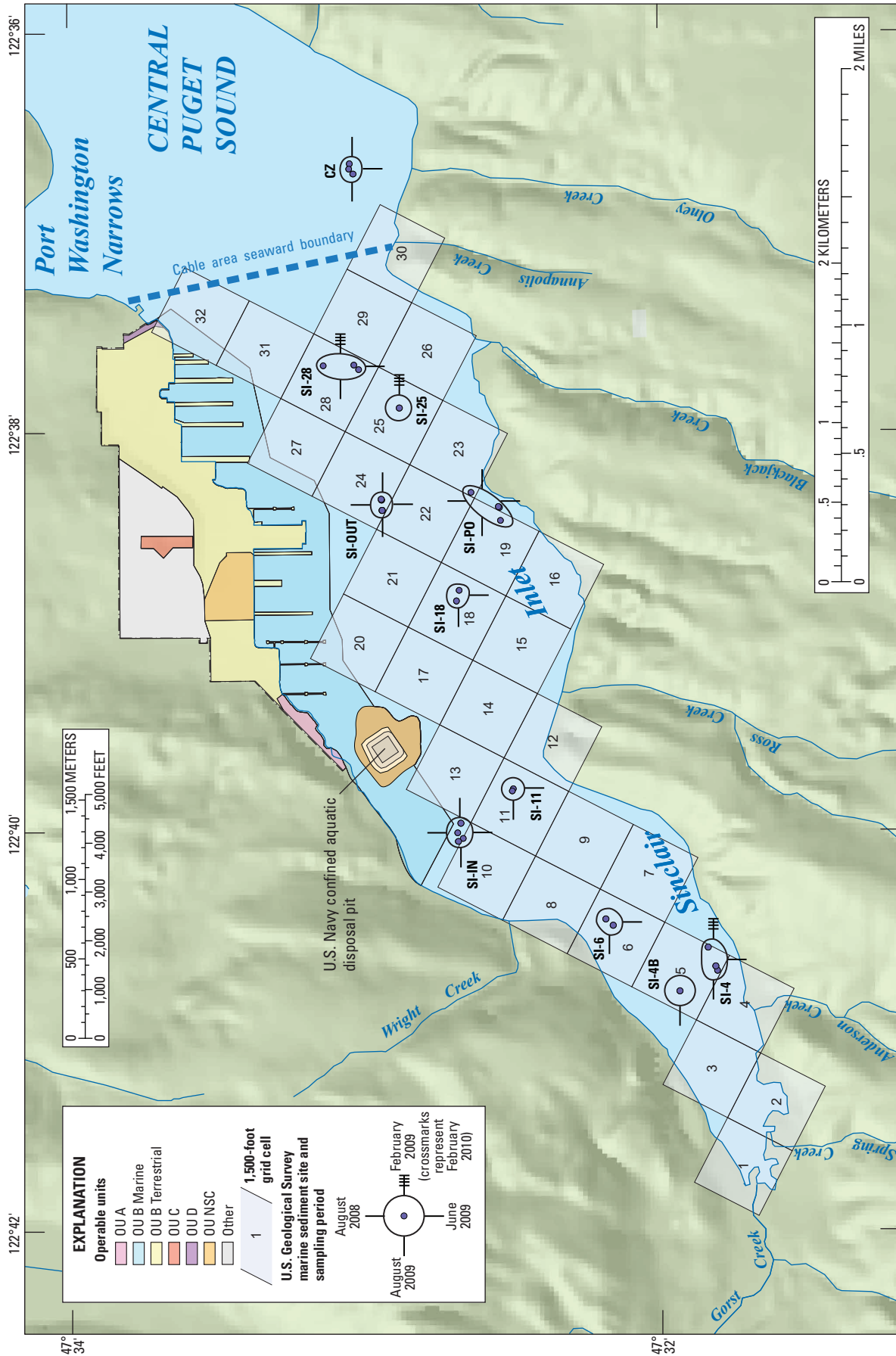
Figure 2.   Marine sediment stations and frequency of sample collection in greater Sinclair Inlet, Kitsap County, Washington, 2008, 2009, 2010.
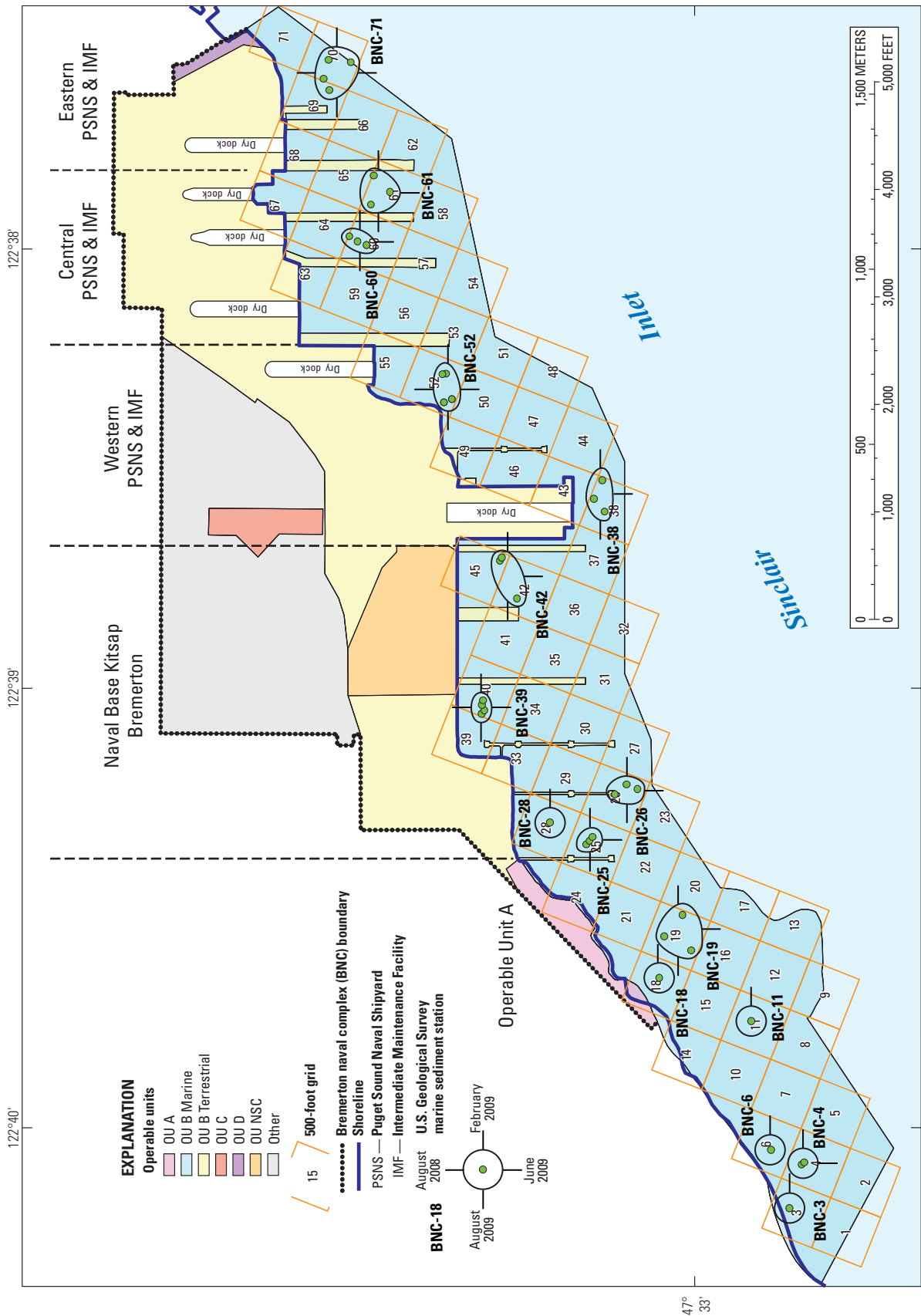
Figure 3.   Marine sediment stations in the OU B Marine, Bremerton naval complex, Kitsap County, Washington, 2008 and 2009.

The sediment of Sinclair Inlet had the highest concentrations of THg and PCBs of all the long-term sediment-monitoring stations in the first Puget Sound-wide sampling effort (Tetra Tech, Inc., 1990). Mercury concentrations in sediment samples collected from Sinclair Inlet and BNC during the Remedial Investigation/Feasibility Study during the 1990s, are summarized in Paulson and others (2010). During screening of marine sediment proposed to be dredged for navigational purposes, a considerable volume of sediment was determined unsuitable for open-water disposal. A Navy confined aquatic disposal pit (fig. 2) was developed in 2000 for disposal of dredge spoils, and dredging of contaminated sediment for CERCLA purposes and was used to fill the excess capacity in the confined aquatic disposal pit. Even after the navigational and CERCLA dredging was completed, the level of THg contamination was of the same magnitude as reported in sediment from Bellingham Bay associated with the Georgia-Pacific chlor-alkali plant and in sediment from Commencement Bay (fig. 1; Paulson and others, 2010). The State of Washington continues long-term monitoring of sediment at one station in Sinclair Inlet and one station in Dyes Inlet, and the U.S. Navy determines THg concentrations at 32 sites in greater Sinclair Inlet and 71 sites with OU B Marine included as part of the monitoring plan outlined in the record of decision (U.S. Environmental Protection Agency, 2000). The second 5-year review for the BNC (U.S. Navy, 2007) identified mercury contamination in marine sediments and groundwater as an ongoing concern.

The ENVironmental inVESTment (ENVVEST) project was developed between Federal, State, and local partners to specifically address the development of Total Maximum Daily Loads for the Sinclair/Dyes Inlet watershed adjacent to PSNS. The final Project Agreement was signed in September 2000 (Washington State Department of Ecology, 2009). The ENVVEST project documented fecal coliform contamination (Cullinan and others, 2007) and measured contaminants of concern, including THg, discharged to Sinclair and Dyes Inlets (Paulson and others, 2010). Since the completion of the USGS marine sampling described in this report and in Paulson and others (2010), the ENVVEST project continues monitoring Sinclair Inlet through an ambient monitoring program (Johnston and others, 2009).

Field Sampling

Sediment, water, and biota were sampled in August 2008 in the three bays discussed in section, "Representative Bays" (fig. 1), three greater Sinclair Inlet stations (fig. 2), and three OU B Marine stations (fig. 3). The Puget Sound embayments, spanning a north-south distance of approximately 70 km, were selected to represent various conditions and tidal exchange regimes that are present across the Puget Sound region. This sample collection was intended to give a regional perspective on the sampling effort in Sinclair Inlet. Starting in September 2008, sampling focused exclusively on stations in Sinclair Inlet. Near-surface water and biota sampling occurred at about monthly intervals. Near-bottom water and sediment sampling occurred on a seasonal basis and were coordinated with the near-surface and biota monthly sampling.

Sediment Sampling

In August 2008, sediment was sampled at three bays discussed in section, "Representative Bays" (fig. 1), three greater Sinclair Inlet stations (Sinclair Inlet-Inner [SI-IN], Sinclair Inlet-Outer [SI-OUT], and Sinclair Inlet-Port Orchard [SI-PO]) (fig. 2), and three OU B Marine stations (BNC-39, BNC-52, and BNC-71) (fig. 3). Sediment was sampled in Sinclair Inlet during four subsequent surface sediment sampling periods (figs. 2 and 3): February 2009 (4 greater Sinclair Inlet stations sites and 16 OU B Marine station); June 2009 (9 greater Sinclair Inlet stations and 11 OU B Marine stations); August 2009 (10 greater Sinclair Inlet stations 10 OU B Marine stations); and February 2010 (3 greater Sinclair Inlet stations). The sampling details and quality-assurance data are reported in Huffman and others (2012). Bottom sediment and overlying water were sampled using a 13.5 × 13.5 × 23-centimeter (cm) deep Eckman-style box corer (Wildlife Supply Company, Buffalo, New York). For each station with incubation experiments, multiple intact sediment cores with at least 10 cm of overlaying water were isolated by sealing an interior sediment core in a 6.35-cm-diameter acrylic core liner with rubber end caps over Parafilm®. Shorter intact cores with less overlying water for reduction-oxidation (redox) sensitive species were isolated in a similar manner. Cores were stored upright in a caddy over ice and transported to the USGS Washington Water Science Center laboratory. The sediment sampling schematic for physical characteristics, mercury, sulfur, and iron species, sediment methylation potential, porewater analyses, tumbling core experiments and incubation experiments are shown in figure 4.
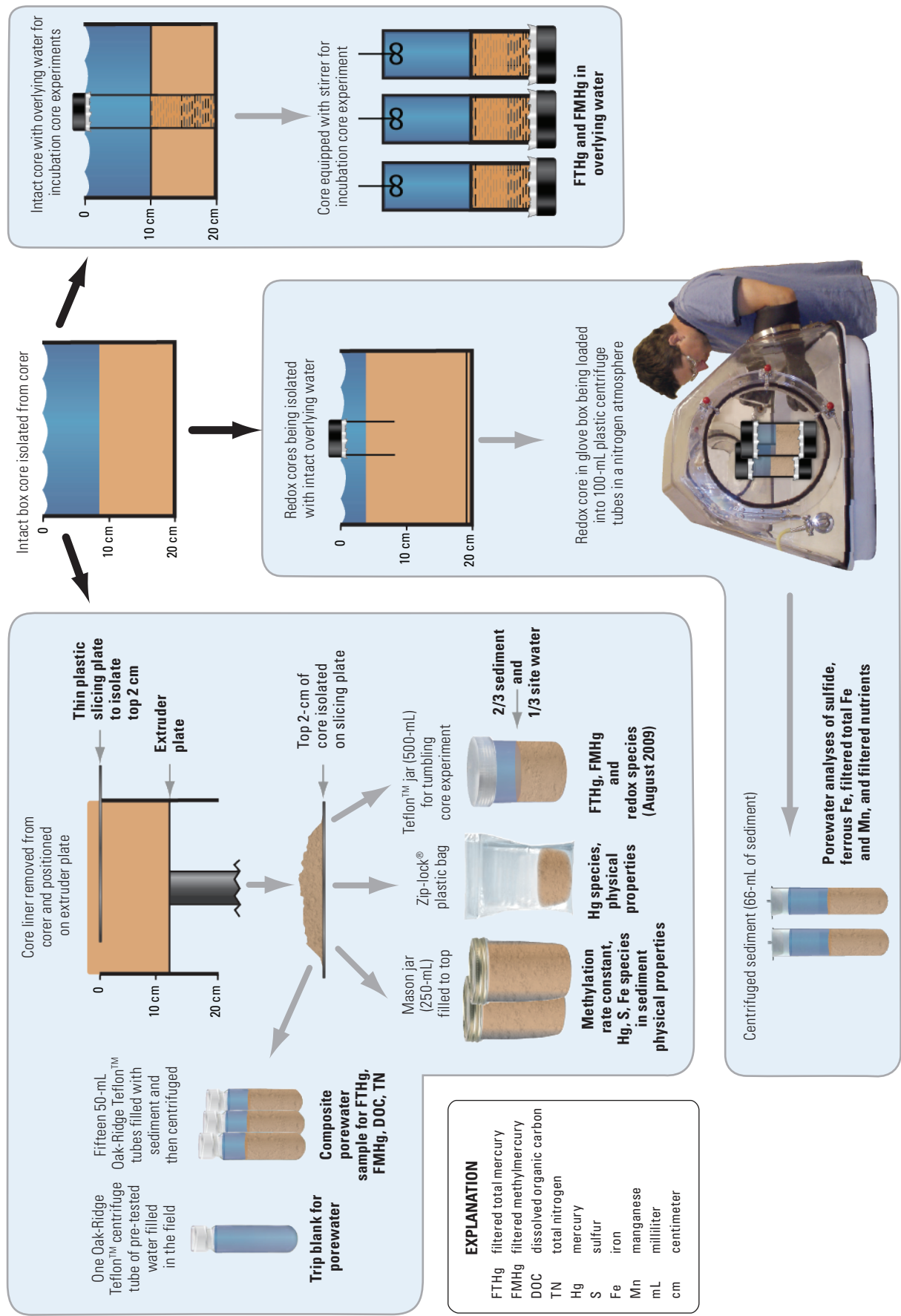
Figure 4.   Sediment methylation potential, porewater analyses, and tumbling-core and incubation experiments.

Sediment in the top 2 cm of the square box corer was collected for all other constituents. Water overlying the sediment from the entire area (13.5 × 13.5 cm) of multiple box cores was removed and saved only for tumbling core experiments. The top 2 cm of sediment from each core was isolated onto an acid-clean sheet of plastic open to the atmosphere. Sediment was collected in (1) two glass jars for analyses of physical characteristics including mercury, sulfur, and iron species, and methylation rates were determined by the USGS National Research Program (NRP) laboratory, Menlo Park, California; and (2) two subsamples for the analyses of mercury species were analyzed by the USGS Wisconsin Mercury Research Laboratory (WMRL). For the subset of stations with incubation experiments, sediment was collected in perfluoroalkoxy copolymer (PFA) beakers for sediment tumbling core experiments and in fifteen 50-milliliter (mL) PFA Oak-Ridge-type centrifuge tubes chilled in the field for extraction of porewater for the analysis of (1) mercury species by the WMRL and (2) dissolved organic carbon (DOC) by NRP laboratory, Boulder, Colorado. In 2009, paired sets of sediment samples were collected randomly from each of two sheets containing the top 2-cm of sediment for duplicate box cores as replicates. All containers were chilled on ice in the field until further processing (except for the subsamples in 2009, which were frozen over dried ice in the field).

Marine Water Sampling

A data sonde (Yellow Springs Instruments Company, Inc.) was used at the three Puget Sound representative bays in August 2008 (fig. 1) to collect water-column profiles of depth, salinity, temperature, dissolved oxygen, turbidity, and fluorescence samples before water chemistry and zooplankton samples were collected. Similarly, vertical profiles were measured monthly at discrete depths in Sinclair Inlet between August 2008 and January 2009. From February 2009 to August 2009, an SBE 19plus (Seabird Electronics., Inc., Bellevue, Washington) conductivity, temperature, and depth (CTD) sensor package was used to collect the same types of samples (for a complete list of dates and locations of sample collection, see Huffman and others [2012], appendix A). Water samples were collected at a minimum of four, occasionally as many as seven, stations in Sinclair Inlet (fig. 5). Near-bottom and near-surface water was collected in August 2008 and February, June, and August 2009 before sediment sampling began.

After collecting profile data, marine water was peristaltically pumped through C-Flex® tubing connected to PFA tubing, which was attached to a polytetrafluoroethylene (PTFE) port that was lowered to the appropriate depth in the following sequence:

1. Raw water was pumped into polyethylene terephthalate glycol (PETG) bottles for mercury species (FTHg, FMHg, PTHg, and PMHg).

2. Water was filtered through Pall Aqua-Prep 0.45 micrometer (μm) pore size, 79-millimeter (mm) diameter, polyester polysulfone disk filter into separate high-density polyethylene (HDPE) bottles for nutrients and total manganese (Mn; acidified in the field).

3. Raw water was pumped in baked amber glass bottles for DOC and total particulate carbon and nitrogen.

4. Raw water was pumped in baked amber glass bottles from the near-surface sites for chlorophyll *a* and isotopes of particulate carbon and nitrogen.

5. Raw water for total suspended solids measurements was pumped in separate HDPE bottles in August and September 2008, after which total suspended solids were measured in every bottle in which PTHg and PMHg samples were collected.

Seawater was processed for analyses of various constituents in a mobile laboratory in August 2008 and at the USGS Washington Water Science Center (WAWSC) laboratory between September 2008 and August 2009. Water-column sampling methods included analysis of nitrate, ammonia, total nitrogen, orthophosphate, total particulate carbon and nitrogen, DOC, and suspended solids.

Zooplankton was collected monthly between August 2008 and November 2009 (except for December 2008) at the BNC-52, SI-IN, SI-PO, and CZ stations. On each sampling date, vertical plankton tows were collected for quantitative analysis at each station using a 0.5 m diameter, 0.1 mm mesh plankton net with an attached TSK flowmeter (Tsurumi Seiki Co., Ltd., North Bend, Washington). The net was lowered to the bottom, depth, data were recorded, and then the net was pulled to the surface at a speed of approximately 0.5 meter per second (m/s). Samples were fixed in 10 percent volume/volume buffered formalin solution. Between three and six additional vertical tows were made with the 0.1 mm mesh net and several vertical tows were made with a 0.75 m diameter, 0.253 mm mesh plankton net to collect live material. The number of net tows depended on the density of organisms observed in the nets. Live specimens were retained and placed on ice in 1-gallon glass jars for less than 24 hours until sample processing began.
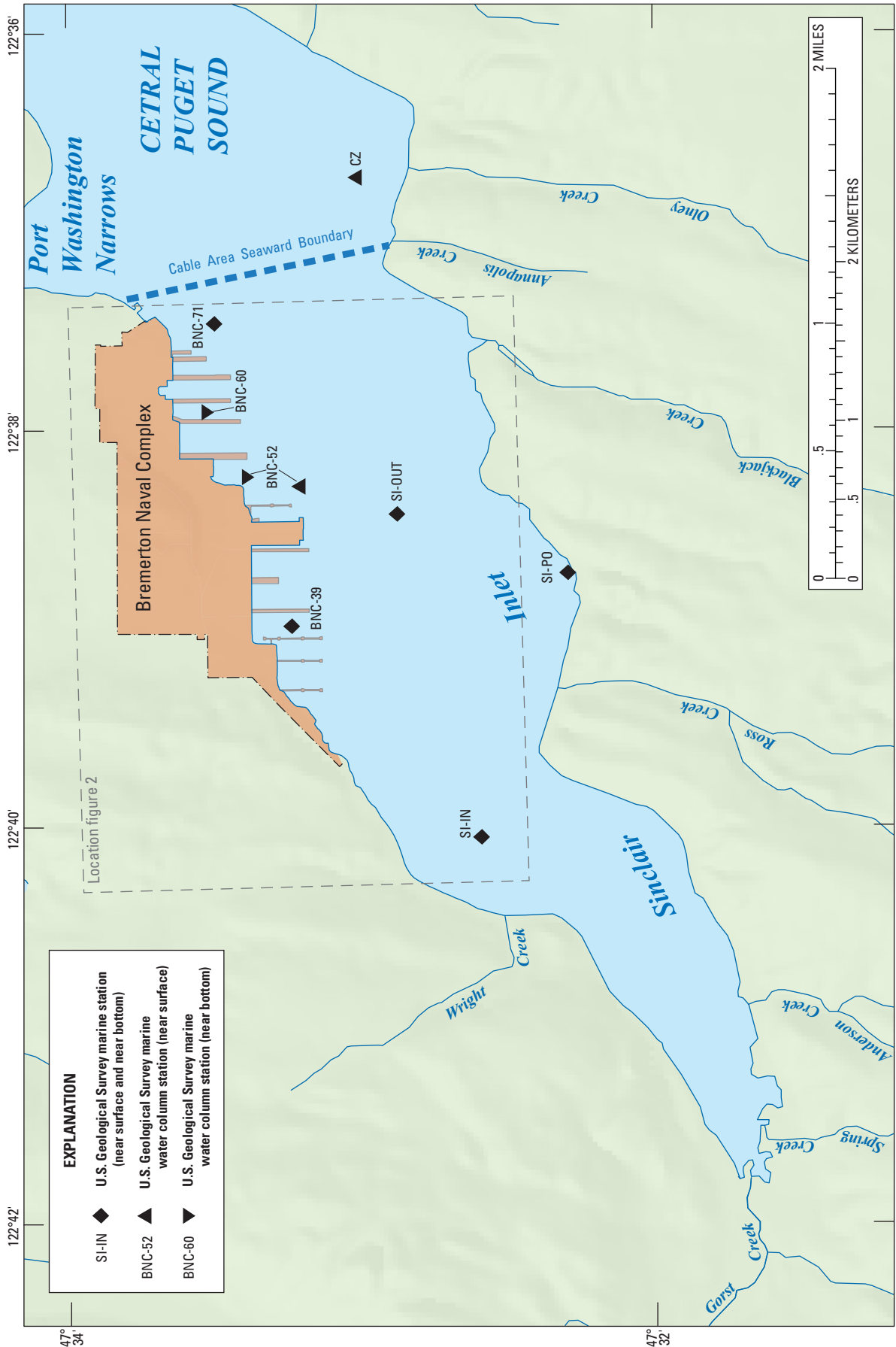
Figure 5.   Locations of marine water-column stations sampled in Sinclair Inlet, Kitsap County, Washington, 2008–10.

Samples of suspended solids and zooplankton from the monthly sampling were also analyzed for stable isotopes to explain the food quality of the suspended solids (particulates) material and its trophic relation to the zooplankton. Stable nitrogen isotope ratios (δ^{15}N) provide a spatially and temporally integrated measure of trophic relations in a food web (that is, primary producers to invertebrates to fish) because δ^{15}N becomes enriched by 2.5–5 parts per thousand between prey and predator (Peterson and Fry, 1987). Stable carbon isotope ratios (δ^{13}C) tend to show little or no enrichment (<1 part per thousand [‰]) with each trophic level, but can identify contributions of different foods (that is, carbon sources), if foods have distinct isotopic signatures (France, 1995).

Statistical Methods

Type II error probability was set at $p<0.05$ for all statistical tests, unless otherwise noted. All data evaluated were reviewed for normality, and appropriate parametric or non-parametric tests were selected based on data distribution characteristics. Data transformations were used, as needed, to meet assumptions of parametric statistics when non-parametric approaches were not, or were less, suitable.

The non-parametric Wilcoxon Rank Sum (WRS) test was used to compare two medians: for sediment data grouped by region (representative bays compared to SI [GSI and OU B Marine combined]) and water column data grouped by depth (surface compared to bottom) in a specific region. The non-parametric Kruskal-Wallis Rank Sum (KWRS) test was used to compare multiple medians for sediment data grouped by sampling period only for the 2009 periods (February, June, and August). In instances where left-censored (less than reporting limit) data existed for a given parameter, summary statistics (medians and interquartile ranges) were calculated using "maximum likelihood estimation" (Helsel, 2005) sub-routines developed by the USGS for the S-Plus statistical platform (ver. 8.1, 2008).

Predictive correlations for surface-sediment mercury metrics were systematically developed. First, potential explanatory X-variables were assessed for normality. In instances where parameters were not normally distributed, various transformations were assessed (that is, ln(X), X^{-1}, X^2, and $X^{1/2}$), and the most appropriate transformation was selected for each parameter either to achieve normality or most closely approach it. In the case of percentage data, the ArcSine square root (ASSR) transformation was used (that is, ArcSine(X%/100)$^{1/2}$). Second, the Akaike Information Criterion (AIC) approach (Akaike, 1974) was used to complete all potential models containing as many as four explanatory variables. The most parsimonious model (that with the minimum number of explanatory variables necessary) was selected after comparing AIC rankings (those with lower AIC scores outrank those with a higher scores), after excluding any candidate models where there was correlation between any two predictor variables exceeding a Pearson correlation coefficient (r) of r greater than +0.7 or r less than -0.7. Third, the model residuals (unexplained error) were examined for normality. Any candidate models with residuals that did not conform to the normal distribution were rejected.

For the sediment porewater parameters, FTHg and FMHg, non-parametric Spearman and Kendal tau correlation analyses were done to determine if correlations were significant. The slope of the parametric Pearson regression is given for datasets in which one or both of the non-parametric tests were significant. A variety of non-parametric analyses of variance was done using geographic and biogeochemical categorical variables. Tukey's multi-comparison method is an ANOVA method to create confidence intervals for all pairwise differences between factor level means resulting in Tukey categories that are significantly different from one another. Water column concentrations greater than the 75th percentile value by more than one interquartile range were considered exceptionally high concentrations and were not included in the ANOVA test. Parameters indicative of accumulation of mercury in aquatic food webs were evaluated using ordinary least squares and stepwise least squares regression techniques. Data were analyzed using TICBO Spotfire S+ (version 8.1, Palo Alto, CA), SYSTAT software (ver. 13, San Jose, CA), or JMP (version 11.2.1; SAS Institute Inc., Cary, NC) statistical software.

II.   Methylation Potential of Mercury in Sediments

By M.C. Marvin-DiPasquale, J.L. Agee, E. Kakouros, L.H. Kieu, D.P. Krabbenhoft, J.F. DeWild, and A.J. Paulson

As part of Task 1 of the Methylation and Bioaccumulation Project (to describe and quantify the biogeochemical cycling of mercury throughout Sinclair Inlet), the study focused on four primary objectives associated with surface sediment. The primary tasks involved (1) quantifying mercury species concentrations; (2) quantifying methylmercury production potential (MPP) rates; (3) examining the extent to which mercury species concentrations and MPP rates vary spatially and seasonally; and (4) examining these spatial and seasonal trends in terms of the sediment carbon, sulfur, and iron biogeochemistry. Sediment sampling sites and sampling periods are shown in figures 2 and 3.

Although net MHg production reflects the balance of gross MHg production and degradation (Marvin-DiPasquale and Agee, 2003; Marvin-DiPasquale and others, 2003), the gross production of MHg is ultimately a function of both the activity of the inorganic-mercury (II) (within the mercury methylating microbial community) and the availability of Hg(II) to those microbes (Marvin-DiPasquale, Lutz, and others, 2009). In terms of controls on the activity of the Hg(II)-methylating microbial community, the factors most commonly cited are the availability of electron acceptors (Gilmour and others, 1992; Kerin and others, 2006), electron donors (that is, labile organic matter; Lambertsson and Nilsson, 2006), and temperature (Heyes and others, 2006). Less is known about the controls on Hg(II) availability to the resident community of Hg(II)-methylating bacteria; however, the specific chemical forms (species) of mercury compounds (Benoit and others, 1999) and dissolved organic matter (Dong and others, 2010; Slowey, 2010) have been cited as playing important roles in this process. In the current study, an attempt was made to determine which environmental variables exert the strongest control on the activity of the Hg(II)-methylating community in surface sediment (as assessed by stable-isotope incubation-derived measurements of methylmercury

production rate [k_{meth}]) and on the availability of Hg(II) to those microbes (as assessed by the sediment reactive inorganic mercury [SRHg] metric). Two primary drivers of MHg production, as methylation rate (k_{meth}) and methylmercury production potential (MPP), both vary spatially (between OU B Marine, GSI, and at a subset of representative bays outside of the Sinclair Inlet) and seasonally (February, June, and August 2009).

Sediment Laboratory Methods

Field samples were subsampled for specific analytes under anoxic conditions in a nitrogen gas (N_2) flushed glove bag. Except for August 2008, when most sub-sampling was done at a local off-site staging area within hours of sample collection, sediment was shipped to the USGS Menlo Park, California, laboratory and subsampled within 1–6 days (median = 2 days, n = 75) from the time of field collection. Unless otherwise noted in Huffman and others (2012), samples typically were homogenized in a large glass bowl with a PTFE spatula. Quality-assurance data presented in Huffman and others (2012) indicate adequate, but occasionally high, variability between homogenized replicates, with THg having 0.4, 4.2, and 59 for minimum relative percent difference (RPD), median RPD, and maximum RPD, respectively, and MHg having 1, 17, and 70 RPDs, respectively. Details about sediment initial subsampling, preservation, and analysis are available in Huffman and others (2012). All sediment parameters analyzed as part of this study, along with analyte names and abbreviation used for each in this text, and a citation for the full method details are listed in table 1. All surface sediment laboratory analyses described in "Methylation Potential of Mercury in Sediments" were completed at the USGS NRP laboratory in Menlo Park, California, unless otherwise indicated.

Mercury Species and Mercury Methylation

For sediment total mercury (STHg), sediment was first digested in concentrated hydrochloric acid (HCl) and nitric acid. The digestate was subsequently subsampled, chemically reduced with tin-chloride, and THg quantified by cold-vapor atomic fluorescence using a Tekran® 2600 automated total mercury analyzer according to U.S. Environmental Protection Agency method 1631 (U.S. Environmental Protection Agency, 2002). For SRHg, thawed sediment was transferred to an

Table 1.   Methods summary and abbreviations used for sediment parameters.

Parameter abbreviation	Parameter name	Method citation
Mercury parameters		
STHg	Sediment total mercury	(Marvin-DiPasquale and others, 2011)
SMHg	Sediment methylmercury	(Marvin-DiPasquale and others, 2011)
$Hg(II)_R$	Inorganic reactive mercury (sediment)	(Marvin-DiPasquale and others, 2011)
k_{meth}	Methylmercury (MHg) production potential rate constant	(Marvin-DiPasquale and others, 2011)
MPP	Methylmercury production potential rate (calculated)	(Marvin-DiPasquale and others, 2011)
Non-mercury parameters		
AVS	Acid-volatile sulfur	(Marvin-DiPasquale and others, 2008)
TRS	Total reduced sulfur	(Marvin-DiPasquale and others, 2008)
$Fe(II)_{AE}$	Acid-extractable ferrous iron [Fe(II)]	(Marvin-DiPasquale and others, 2008)
$Fe(III)_a$	Amorphous (poorly crystalline) ferric iron [Fe(III)]	(Marvin-DiPasquale and others, 2008)
$Fe(III)_c$	Crystalline ferric Iron [Fe(III)]	(Marvin-DiPasquale and others, 2008)
Fe_T	Total iron (calculated; sum of measured three iron fractions)	(Marvin-DiPasquale and others, 2008)
%LOI	Percentage of weight loss on ignition	(Marvin-DiPasquale and others, 2008)
BD	Bulk density	(Marvin-DiPasquale and others, 2008)
%FINES	Percent fines, grain size (percent, less than 63 micrometers)	(Matthes and others, 1992)
E_h	Oxidation-reduction potential	(Marvin-DiPasquale and others, 2008)
pH	pH units	(Marvin-DiPasquale and others, 2008)

N_2-flushed bubbler containing 0.5 percent HCl, reduced with tin-chloride, and purged with N_2 for 15 minutes, and the resulting elemental mercury was collected on an in-line gold trap and subsequently quantified by using cold-vapor atomic fluorescence spectrometry (CVAFS) (Marvin-DiPasquale and Cox, 2007). Sediment methylmercury (SMHg) was first extracted with a solution of 25 percent potassium hydroxide (KOH) in methanol (Xianchao and others, 2005); SMHg is used here to denote MHg extracted from sediments, whereas PMHg is used to denote MHg measured on particles from collected from the water column. The extraction solution subsequently was subsampled, ethylated with sodium tetraethyl borate in a sealed vessel, and quantified for SMHg using a MERX (Brooks Rand, Seattle, Washington) automated MHg analyzer.

Surface sediment Hg(II)-methylation rate constants (k_{meth}) were assessed with 4–5 hour bottle incubation core experiments composed of native sediment and site water (fig. 4), amended with short-term (4–5 hour) stable isotope amendments (Huffman and others, 2012). Incubation temperature was set at ±1 degree Celsius (°C) of the average sediment temperature measured in the field at the time of collection for all sites sampled. The labeled [200]Hg-methylmercury, an isotopically enriched form of mercury, was extracted with KOH/methanol and quantified using isotope-dilution inductively coupled plasma with mass spectrometry. The MPP rates were subsequently calculated using independently measured k_{meth} and SRHg data for each site (referred to as Hg(II)R in Huffman and others [2012]).

Physical Characteristics of Sediment and Speciation of Iron and Sulfur

Acid-volatile sulfur (AVS) concentration, a measure of substances that generate hydrogen sulfide (H_2S) gas upon HCl addition, was measured on wet sediment collected during only August 2008, using hot acid distillation, H_2S–trapping, and colorimetric quantification of sulfide (Marvin-DiPasquale and others, 2008). Total reduced sulfur (TRS) concentration was measured on all sediment samples using a single-step hot acid, chromium-reduction distillation with H_2S-trapping and colorimetric quantification of sulfide (Marvin-DiPasquale and others, 2008). Three iron species were measured by chemical extraction and colorimetric quantification (Marvin-DiPasquale and others, 2008), which included acid-extractable ferrous iron ($Fe(II)_{AE}$), amorphous (poorly crystalline) ferric iron ($Fe(III)_a$), and crystalline ferric iron ($Fe(III)_c$). The sum of these three iron fractions is calculated as a measure of total iron ($Fe_T = Fe(II)_{AE} + Fe(III)_a + Fe(III)_c$). The percentage of $Fe(II)_{AE}$ relative to Fe_T (%$Fe(II)_{AE} = Fe(II)_{AE}/Fe_T \times 100$) also was calculated. Sediment bulk density (BD), dry weight, porosity, and organic content (as percentage of weight loss on ignition; LOI) were measured in sequence from a single sediment subsample (Marvin-DiPasquale and others, 2008). Sediment grain size, assessed as the sand/silt split and expressed as percentage of fines (<63 µm), was done by wet sieving and subsequent drying (Matthes and others, 1992). Sediment redox potential and pH were measured by electrode (Marvin-DiPasquale and others, 2008).

Regional Analysis of Sinclair Inlet Compared to the Representative Bays

Statistical results of the 2008 (reconnaissance sampling) sediment parameters from the four representative bays (fig. 1) located outside of Sinclair Inlet (CZ, HH, LB, BI) for the comparisons with the stations located inside of Sinclair Inlet (see fig. 2) and the sites within the Operable Unit shown in figure 3. Concentrations of STHg were significantly greater in the Sinclair Inlet stations than those measured at the representative stations, with the median value for the greater Sinclair Inlet stations (305 ng/g) being approximately six-fold greater than the median value for the representative stations (55 ng/g). Similarly, median SRHg concentrations were eight-fold greater in the Sinclair Inlet stations (0.57 ng/g) compared to the representative bays (0.07 ng/g), with an eight-fold higher median concentration in the Sinclair Inlet (table 2). The only other significant difference detected was for SMHg as a percentage of STHg, for which the median value was more than five-fold greater for the representative bays (3 percent of STHg as SMHg) compared to the Sinclair Inlet stations with 0.6 percent of STHg as SMHg. That is, the percentage of sediment total mercury in the methyl form in representative bays was much higher than in Sinclair Inlet. In contrast, a number of key mercury parameters were not significantly different inside than outside of Sinclair Inlet during the 2008 reconnaissance sampling, including SMHg concentrations, SRHg as a percentage of STHg, the experimentally derived rate constants for Hg(II)-methylation (k_{meth}), and MPP rates. Furthermore, none of the non-mercury sediment parameters assayed for August 2008 were significantly different inside than outside of Sinclair Inlet (see table 2, not all parameters shown).

Although more THg (and SRHg) is in the sediment collected from Sinclair Inlet compared to representative bays outside of Sinclair Inlet, this limited dataset provides little evidence that SMHg concentrations or production rates vary significantly inside than outside of Sinclair Inlet. The one exception is MHg as a percentage of STHg, which has been considered a measure of the Hg(II)-methylation efficiency in some instances (Gilmour and others, 1998; Krabbenhoft and others, 1999; Domagalski, 2001). These results imply that as a group, the sediment associated with the representative bays had a higher efficiency for MHg production than did stations in Sinclair Inlet. However, that interpretation was not consistent with the MHg concentrations or measured MPP rates. It is more likely that both groupings had a similar propensity for MHg production, based on the activity of the resident Hg(II)-methylating microbes (as implied by similar k_{meth} values) and similar MHg concentrations. However, because the THg concentrations inside Sinclair Inlet were so much greater than in the representative bays, the calculated percentage of MHg (that is, percentage of MHg = [MHg]/[THg] × 100 percent) values were much lower for stations inside Sinclair Inlet.

Table 2.   Wilcoxon Rank-Sum test results for all Sinclair Inlet stations and representative bays sampled during August 2008, Puget Sound, Washington.

[Parameter definitions are given in table 1. Bold values indicate statistically significant difference. The non-parametric Wilcoxon Rank Sum comparison of medians included all eleven sites sampled during the August 2008 reconnaissance sampling event, for data grouped by greater Sinclair Inlet and Bremerton naval complex sites (combined; n=7) and by representative sites located outside of the Sinclair Inlet (n=4). The first quartile (25th percentile), median (50th percentile), third quartile (75th percentile) are shown. **Wilcoxon Rank Sum: ***,** significant differences between sediment mercury parameter groupings at the probability levels of $p < 0.05$; NS, non-significant differences. All non-mercury sediment parameter comparisons were NS. **Abbreviations:** ng/g, nanogram per gram; (pg/g)/d, picogram per gram per day]

Parameter (units)		Representative bays			Greater Sinclair Inlet and Bremerton naval complex sites			Wilcoxon Rank Sum
		25th percentile	Median	75th percentile	25th percentile	Median	75th percentile	
STHg[1]	(ng/g) dry weight	15	**55**	94	191	**305**	659	***
SRHg	(ng/g) dry weight	0.04	**0.07**	0.12	0.38	**0.57**	0.60	***
%Hg(II)$_R$	(percentage of STHg)	0.16	0.18	0.22	0.08	0.15	0.29	NS
SMHg	(ng/g) dry weight	0.88	1.90	2.93	1.47	1.79	3.81	NS
%SMHg	(percentage of STHg)	2.71	**3.29**	5.22	0.51	**0.61**	1.12	***
k_{meth}[1]	(day)	0.007	0.016	0.024	<0.0014	0.011	0.014	NS
MPP rate[1]	([pg/g]/d) dry weight	0.3	1.7	3.1	<0.84	2.7	6.9	NS

[1] Parameter contained left-censored (less than) data. Interquartile range and medians calculated using maximum likelihood estimate statistics (Helsel, 2005).

Spatial Analysis of Sediment from Bremerton Naval Complex Compared to Greater Sinclair Inlet

Comparing BNC stations to GSI stations across all sampling dates, STHg and SRHg concentrations were significantly greater in the BNC than in the GSI sediment stations. Non-mercury sediment parameters, TRS, Fe(II)$_{AE}$, Fe$_T$, the percentage of Fe(II)/Fe$_T$, the percentage of fines (<63 μm), E$_h$, and pH, also were greater in the BNC than in the GSI sediment (table 3), based on Wilcoxon rank-sum test. The only sediment mercury parameter that was significantly lower for BNC than the GSI was the percentage of SMHg/STHg, as were non-mercury parameters Fe(II)$_a$

and field temperature. No other sediment mercury parameters (SMHg, the percentage of SRHg/STHg, k$_{meth}$, and MPP rate) or non-mercury parameters showed significant difference between BNC stations and GSI stations. These results suggest that although the absolute concentration of STHg and SRHg is higher in the BNC, than in the GSI, the MPP rate and SMHg concentration is not. The higher STHg and SRHg content in the BNC may be partially due to smaller sediment particles (higher percentage of fines <63 μm) in this area; an increase in STHg concentration typically is observed as particle size decreases (Bengtsson and Picado, 2008; Fleck and others, 2011) due to the increase in the ratio of particle surface area to volume.

Table 3.   Wilcoxon Rank-Sum test results comparing sediment mercury and non-mercury parameters from Operable Unit B Marine and Greater Sinclair Inlet stations, Kitsap County, Washington.

[Parameter definitions are given in table 1. Bold values indicate statistically significant difference. The non-parametric Wilcoxon Rank Sum comparison of medians included all eleven sites sampled during the August 2008 reconnaissance sampling event, for data grouped by greater Sinclair Inlet and Bremerton naval complex sites (BNC; n=38) and greater Sinclair Inlet (n=32). The first quartile (25th percentile), median (50th percentile), third quartile (75th percentile) are shown along with all results from all mercury metric comparisons. **Wilcoxon Rank Sum:** ***, significant differences between sediment mercury parameter groupings at the probability levels of $p < 0.05$; NS, non-significant differences. **Abbreviations:** °C, degrees Celsius; μm, micrometer; μmol/g, micromole per gram; mg/g, milligrams per gram; ng/g, nanogram per gram; n.c., not calculated due to the number of left-censored (less than); pg/g, picogram per gram; (pg/g)/d, picogram per gram per day; <, less than]

Parameter (units)		Bremerton naval complex			Greater Sinclair Inlet			Wilcoxon Rank Sum
		25th percentile	Median	75th percentile	25th percentile	Median	75th percentile	
Sediment mercury parameters								
STHg	(ng/g) dry weight	510	**674**	756	152	**457**	649	***
Hg(II)$_R$[1]	(ng/g) dry weight	0.32	**0.42**	0.49	0.19	**0.28**	0.39	***
%Hg(II)$_R$[1]	(percentage of STHg)	0.050	0.063	0.093	0.045	0.058	0.126	NS
SMHg	(ng/g) dry weight	1.95	2.69	4.09	0.95	2.86	5.29	NS
%SMHg	(percentage of STHg)	0.28	**0.42**	0.66	0.56	**0.90**	1.12	***
k$_{meth}$[1]	(day)	**n.c.**	<0.00079	0.012	<0.00077	0.0048	0.016	NS
MPP rate[1]	([pg/g]/d) dry weight	**n.c.**	<0.21	3.60	<0.01	0.84	5.23	NS
Sediment non-mercury parameters								
TRS	(μmol/g) dry weight	218	**249**	290	80	**163**	277	***
Fe(II)$_{AE}$	(mg/g) dry weight	3.3	**4.0**	4.5	1.5	**3.2**	3.8	***
Fe(III)$_a$[1]	(mg/g) dry weight	0.010	**0.012**	0.030	0.01	**0.02**	0.11	***
Fe$_T$	(mg/g) dry weight	3.6	**4.6**	5.1	2.7	**3.5**	4.8	***
%Fe(II)/Fe$_T$	(percent)	80	**91**	99	63	**81**	99	***
%FINES	(percent, <63 μm)	72	**84**	87	36	**74**	86	***
E$_h$ laboratory	(millivolt)	-17	**1**	24	-69	**-16**	4	***
pH units	(pH units)	7.13	**7.20**	7.25	7.01	**7.09**	7.17	***
Field temperature	(°C)	7.6	**11.3**	13.8	11.3	**13.5**	7.17	***

[1]Parameter contained left-censored (less than) data. Interquartile range and medians calculated using maximum likelihood estimate statistics (Helsel, 2005).

Seasonal Analysis

A non-parametric statistical analysis (KWRS test) of mercury and non-mercury sediment parameters was done on data grouped by month (February, June, and August 2009) for all dates and all stations in the BNC and GSI (combined; table 4). Significant temporal trends were indicated in all mercury (Hg) parameters except in STHg and SRHg concentrations. Overall, SMHg concentration, percentage of SMHg/STHg, k_{meth}, and MPP rates were lowest in February and higher in June and August. Temperature also was low during these months, which suggests the ever-present influence of temperature on microbial Hg(II)-methylation. Additionally, surface sediment organic content (as percentage of LOI) was highest in June (median = 10.5 percent) and similar in February and August (medians = 8.6 percent and 8.9 percent, respectively). This temporal trend may partially reflect the deposition of phytodetritus associated with a spring bloom in phytoplankton (see section, "Release of Mercury Species from Sediment to the Water Column"), which was evident by a spike in chlorophyll *a* in the water column with a concentration of 16 µg/L during April. However, much larger chlorophyll *a* spikes were seen in the late summer and autumn (August and September) in

Sinclair Inlet (see section, "Release of Mercury Species from Sediment to the Water Column," and Huffman and others, 2012). Thus, the combination of increasing temperature and the episodic loading of labile organic material to the sediment surface during spring and summer likely drive the higher overall SMHg concentrations and MPP rates in this system. A more frequent temporal sediment sampling program (for example, monthly) would be required to further resolve the relative importance of temperature compared with organic loading on SMHg production dynamics in the Sinclair Inlet, see figure 6.

Figure 6.   (*A*) Sediment methylmercury concentration and (*B*) sediment methylmercury production potential rates at Sinclair Inlet stations, Washington, 2009.

Table 4.   Kruskal-Wallis Rank Sum test results comparing combined data by month for all Sinclair Inlet stations, Kitsap County, Washington, 2009.

[Parameter definitions are given in table 1. Bold values indicate statistically significant difference. The non-parametric Kruskal-Wallis Rank Sum test comparison of medians grouped by sampling season (month) [February (n=23), June (n=20) and August (n=27)] for all dates and all sites within both Bremerton Naval Complex and greater Sinclair Inlet (combined). The first quartile (25th percentile), median (50th percentile), third quartile (75th percentile) are shown along with all results from all mercury metric comparisons. **Kruskal-Wallis Rank Sum:** ***, significant differences between groupings at a probability level of $p < 0.05$; NS, non-significant differences. Only significant results for non-mercury metrics are shown. **Abbreviations:** d, day; °C, degrees Celsius; g/cm^3, gram per cubic centimeter; µmol/g, micromole per gram; ng/g, nanogram per gram; n.c., not calculated due to the number of left-censored (less than) data; nanogram per gram; pg/g, picogram per gram; (pg/g)d, picogram per gram per day; <, less than]

Parameter (units)	February			June			August			Kruskal-Wallis Rank Sum
	25th percentile	Median	75th percentile	25th percentile	Median	75th percentile	25th percentile	Median	75th percentile	
Sediment mercury parameters										
STHg (ng/g) dry weight	442	658	768	461	530	712	240	535	649	NS
Hg(II)$_R$[1] (ng/g) dry weight	0.23	0.33	0.40	0.23	0.35	0.46	0.28	0.43	0.58	NS
%Hg(II)$_R$[1] (percentage of STHg)	0.04	**0.05**	0.06	0.05	**0.06**	0.08	0.06	**0.09**	0.13	***
SMHg (ng/g) dry weight	1.32	**2.11**	2.33	1.55	**4.53**	6.07	1.86	**3.63**	4.41	***
%SMHg (percentage of STHg)	0.24	**0.30**	0.44	0.41	**0.79**	1.12	0.57	**0.83**	1.13	***
k_{meth}[1] (day)	n.c.	**<0.00077**	0.0057	<0.00082	**0.0069**	1.12	<0.0010	**0.0049**	0.017	***
MPP rate[1] ([pg/g]/d) dry weight	n.c.	**<0.014**	1.83	<0.22	**1.00**	4.30	4.30	**1.66**	7.21	***
Sediment non-mercury parameters										
BD (g/cm^3) wet weight	1.16	**1.20**	1.28	1.11	**1.12**	1.12	1.12	**1.15**	1.21	***
TRS (µmol/g) dry weight	182	**271**	300	196	**262**	262	109	**197**	243	***
% LOI (percentage of dry weight loss on ignition)	5.9	**8.6**	9.4	8.5	**10.5**	10.5	7.7	**8.9**	9.9	***
E_h laboratory (millivolt)	-6	**29**	84	-57	**-6**	0	-54	**-17**	3	***
Field temperature (°C)	7.5	**7.6**	8.0	11.3	**11.5**	11.5	13.9	**14.1**	14.6	***

[1]Parameter contained left-censored (less than) data. Interquartile range and medians calculated using maximum likelihood estimate statistics (Helsel, 2005).
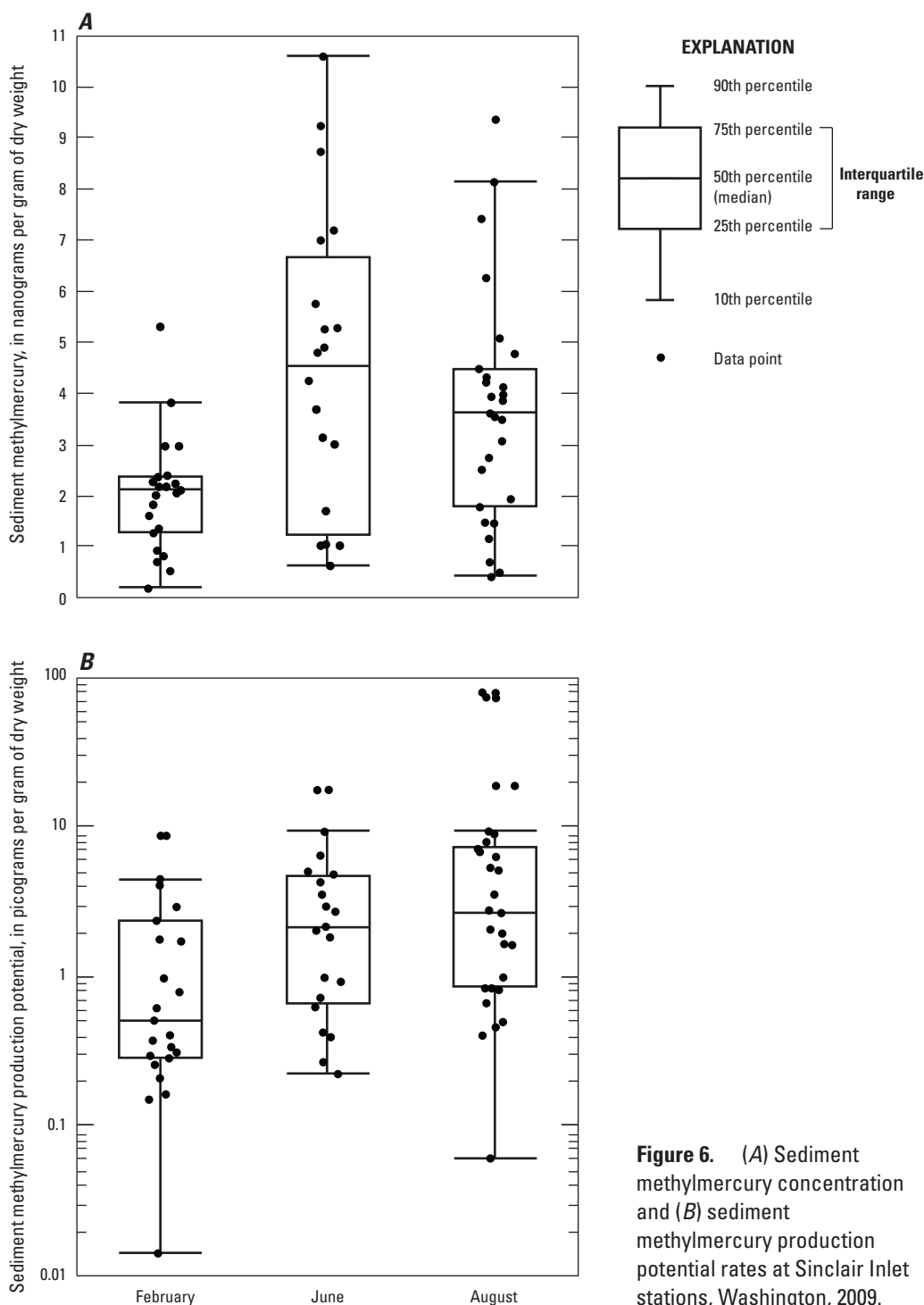
Controls on Gross Methylmercury Production

Gross SMHg production in sediment is essentially a function of the activity of the Hg(II)-methylating microbial community and the availability of Hg(II) to that community, with many factors mediating both terms. To better understand which environmental factors play a significant role in the temporal and spatial variations for surface SMHg concentrations in the current study, the factors most influential in controlling the activity of the Hg(II)-methylating microbial community (as approximated by k_{meth}) and the availability of Hg(II) to that community (as approximated by SRHg concentration) were examined. Then how the calculated MPP rate, which is a function of both k_{meth} and SRHg, compares to SMHg concentration was examined to draw some inferences on the potential role of SMHg degradation, a pathway that was not directly examined in this study.

The best linear regression model describing k_{meth}, based on AIC analysis (comparing all possible models with as many as four explanatory variables), included the sediment variables, temperature (used for incubations), reduction-oxidation (redox as E_h), and percentage of $Fe(II)_{AE}$ (ASSR-transformed). The resulting final regression equation, using data for all stations and sampling periods, accounted for 37 percent of the measured variability in $\ln[k_{meth}]$ (fig. 7). The signs on the respective coefficients indicate that $\ln[k_{meth}]$ is negatively related to sediment E_h and positively related to incubation temperature and the percentage of $Fe(II)_{AE}/Fe_T$, the latter reflecting the extent to which Fe(III) has been reduced to Fe(II), presumably largely by microbial iron reduction (Marvin-DiPasquale and others, 2014). Because concentration data (for example, $Fe(II)_{AE}$ and Fe_T data used to calculate the percentage of $Fe(II)_{AE}/Fe_T$ are not microbial rate data, the positive correlation between k_{meth} and the percentage of $Fe(II)_{AE}/Fe_T$ cannot be inferred. As revealed by the best regression model, but rather indicates that k_{meth} is a direct function of microbial iron-reduction in the sites sampled for this study. However, we note that various other candidate models that included sediment TRS (a metric often associated with microbial sulfate reduction [Marvin-DiPasquale and others, 2014]) were ranked lower than the best model presented here. A recent study of freshwater wetland sediment (Marvin-DiPasquale and others, 2014) determined that k_{meth} was positively correlated with sediment TRS concentration, as well as with net iron-reduction as assessed by the seasonal change in $Fe(II)_{AE}$ concentration between sampling dates.

A similar AIC competitive model approach was used to develop a predictive function for ln-transformed sediment reactive inorganic mercury (ln[SRHg]). The resulting top model, using data for all stations and sampling events, calculated 45 percent of the measured variability in ln[SRHg] with sediment E_h and STHg (ln-transformed) as positive variables and sediment wet bulk density (ln-transformed) (ln[BD]) as a negative variable (fig. 7). The negative correlation between sediment SRHg and bulk density is

because sediment bulk density is a function of both sediment organic content and sediment grain size. This is strongly negatively correlated with both r = -0.94 and r = -0.88, respectively (not shown) and because sediment SRHg exhibited weak positive correlations with sediment organic content (r = 0.46) and grain size (r = 0.50) for the complete dataset. Thus, sediment with a low bulk density tends to have high organic content and (or) a high percentage of fine grained particles (as opposed to sand).

Because SRHg concentrations were significantly higher in the BNC and Sinclair Inlet stations (combined), compared to the representative bays during August 2008 (table 2), another model was run that excluded the representative bays to determine if the same parameters caused the variation in SRHg concentration in the BNC and GSI stations, but not the representative bays. The resulting best-fit equation was less strong (model $R^2 = 0.37$, not shown), with ln[SRHg] being a positive function of both sediment E_h and ln[BD]. Thus, sediment redox potential plays a consistent role in estimating both the activity of the Hg(II)-methylating community and the availability of Hg(II) to those microbes. However, as indicated
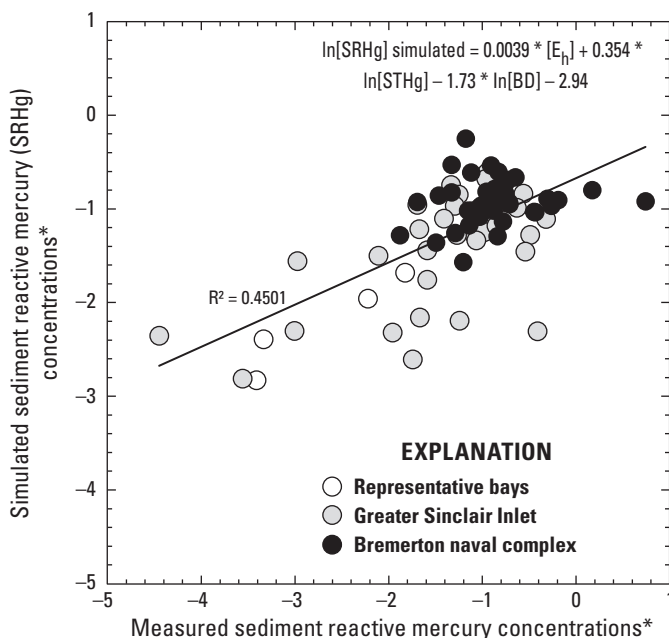
Figure 7. Simulated and measured sediment methylation rate constant from the sediment reactive mercury pool developed for representative bays, Bremerton naval complex and greater Sinclair Inlet, Kitsap County, Washington, 2008–09. (Model-simulated values for ln-transformed sediment reactive mercury (ln[SRHg]) are based on the least squares multiple linear regression equation, with X variables: sediment redox (E_h), ln-transformed sediment total mercury concentration (ln[STHg] and ln-transformed sediment bulk density (ln[BD]).) Data include all samples collected from study area. The coefficient of determination (R^2) for the regression is given.

by the multiple regression equations, as sediment E_h increases (sediment becomes more oxidized), SRHg increases (positive E_h coefficient in fig. 7). This partially explains why estimating SMHg concentration or MPP rates, based on sediment redox conditions alone, is difficult if not impossible.

The observation that the availability of Hg(II) for Hg(II)-methylation can be affected by sediment redox conditions or redox-sensitive sediment constituents has been previously reported. In a study of eight diverse stream systems, sediment SRHg concentration was a positive function of sediment $Fe(III)_a$ concentration, whereas the percentage of SRHg was a negative function of sediment TRS concentration (Marvin-DiPasquale, Lutz, and others, 2009). In studies of freshwater wetlands in the Central Valley of California, the percentage of sediment SRHg/STHg increased with increasing sediment E_h values (Marvin-DiPasquale, Alpers, and Fleck, 2009), whereas the concentration of sediment SRHg decreased with increasing sediment AVS (Marvin-DiPasquale, Alpers, and Fleck, 2009) and TRS (Marvin-DiPasquale and others, 2014) concentrations. These previous reports are consistent with the current study, where sediment E_h was a positive term in the multivariable model describing SRHg concentration and where the percentage of SRHg/STHg decreased with increasing sediment AVS concentration during August 2008 ($R^2 = 0.84$, n=11), which was the only sampling event for which AVS was assayed (fig. 8).

Because Hg(II) availability is an important factor that mediates SMHg production, and because SRHg concentration is partially a
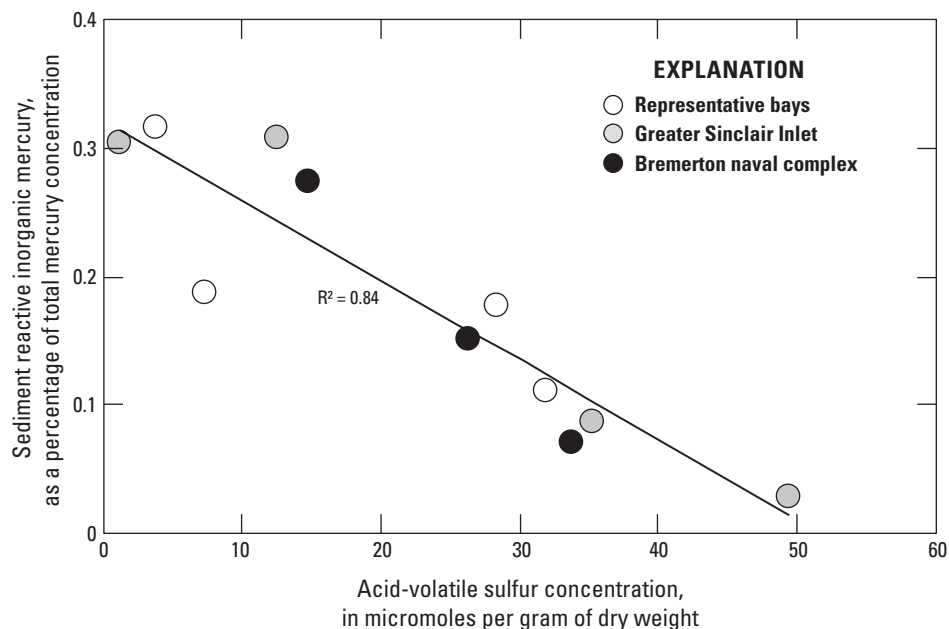
Figure 8.   Sediment reactive inorganic mercury [SRHg] as a percentage of sediment total mercury concentration compared to acid-volatile sulfur concentration for all stations sampled in Sinclair Inlet, Kitsap County, Washington, during August 2008.

function of STHg concentration across all stations and dates, then the next question is "What controls STHg concentration across the study stations?" Using the AIC competitive model approach, the top ranking model included sediment bulk density only and explained 69 percent of the STHg concentration for all BNC and GSI sites (fig. 9). Sediment bulk density reflects (and is negatively correlated with) both sediment organic content and grain size. Lower ranking models did include these terms, and both terms have been cited as primary controls of STHg concentration (Horowitz, 1985; Scudder and others, 2009). When the representative bay sites were included, the top model used both sediment TRS and grain size (positive coefficients for both); however, the model residuals (unexplained error) were not normally distributed and the model was rejected.

The top ranked model for SMHg concentration (ln-transformed) included sediment E_h and bulk density, both as negative terms, and explained 83 percent of the variability in SMHg across all BNC and GSI sites (fig. 10). When representative bay sites were included, the top model contained E_h and bulk density as negative terms, but also included STHg as a positive term (model $R^2 = 0.81$, not shown). The negative correlation between SMHg and sediment E_h is consistent with high SMHg concentration at locations with high rates of microbial Fe(III)-reduction and sulfate reduction, which typically are more chemically reducing. The negative correlation between SMHg and sediment bulk density also is consistent with high MHg concentrations at locations with high organic content and (or) a high proportion of fine-grained particulates.
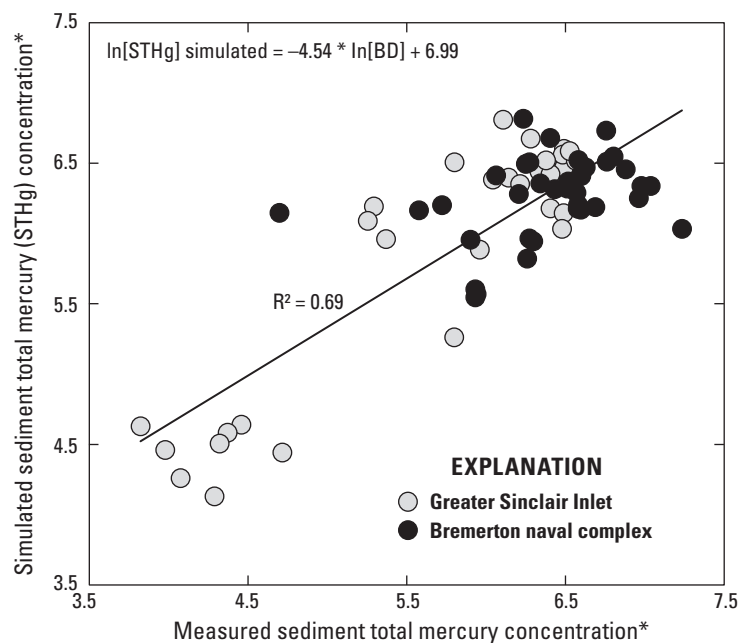
Figure 9. Simulated and measured sediment total mercury concentration, Sinclair Inlet, Kitsap County, Washington. (Simulated values of sediment total mercury (ln-transformed) (ln[STHg]) are based on the least squares linear regression equation, with sediment wet bulk density (ln-transformed) (ln[BD]) as the X variable.) Data include all sampling periods and from all greater Sinclair Inlet and Bremerton naval complex stations.
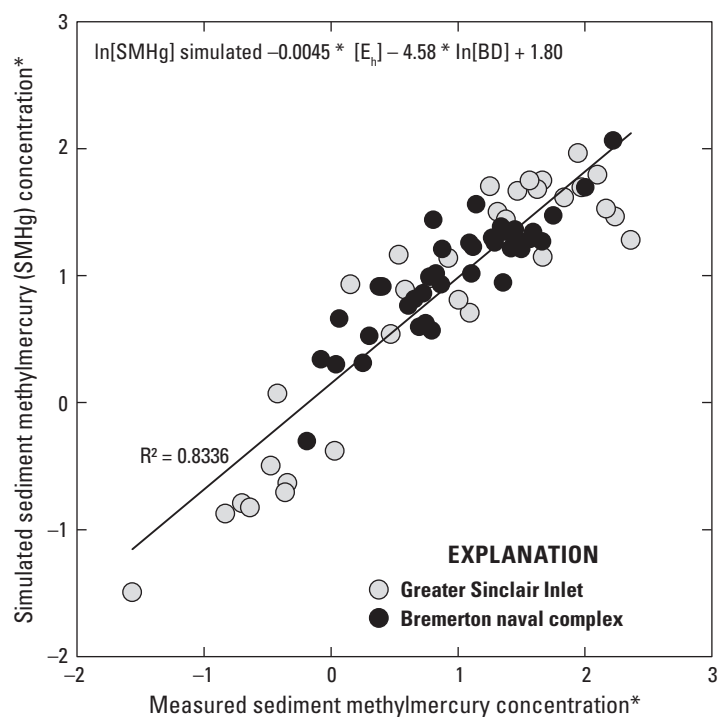
Figure 10. Simulated and measured sediment methylmercury concentration, Sinclair Inlet, Kitsap County, Washington. (Simulated sediment methylmercury concentrations (ln-transformed) (ln[SMHg]) are based on the least squares multiple linear regression equation, with X variables: sediment redox (E_h) and wet bulk density (ln-transformed) (ln[BD]).) Data are from all sampling periods and from all greater Sinclair Inlet and Bremerton naval complex stations.
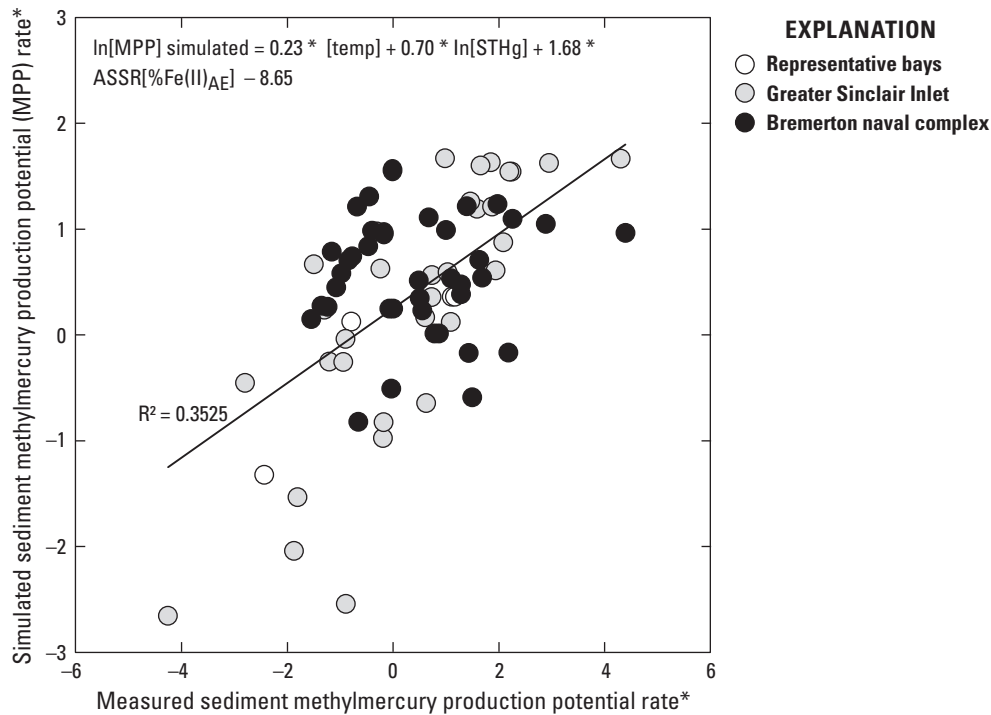
Figure 11.   Simulated and measured sediment methylmercury production potential (MPP) rates, Sinclair Inlet, Kitsap County, Washington. (Simulated values of sediment methylmercury production potential rates (ln-transformed) (ln[MPP]) are based on the least-squares multiple linear regression equation, with X variables: incubation temperature (temp), sediment total mercury (ln-transformed) (ln[STHg]), and percentage of $Fe(II)_{AE}/Fe_T$ (arcsine square root [ASSR] transformed) ($ASSR[\%Fe(II)_{AE}]$).) Data are from all sampling periods and all sites, including representative bays, greater Sinclair Inlet and the Bremerton naval complex stations.

The top ranked model for MPP rates (ln-transformed) included incubation temperature, STHg (ln-transformed), and the percentage of $Fe(II)_{AE}/Fe_T$ (ASSR transformed), with all three positive terms (model $R^2 = 0.35$; fig. 11). When representative bays were excluded from the analysis, the top ranked model for MPP rates (ln-transformed) was only marginally improved (model $R^2 = 0.36$), with X variables of incubation temperature and grain size (as percentage of FINES; ASSR transformed), both as positive terms. Fe_T (square root transformed) as a negative term also was used for all BNC and GSI sites combined (not shown). As for both MPP and SRHg top ranked models, STHg was a dominant explanatory variable only when representative bays were included in the analyses. This reflects the fact that median STHg concentrations were significantly lower for representative bays than all Sinclair Inlet stations (GSI and BNC combined, table 2), although, within the primary study area, median STHg concentrations also were significantly lower for GSI stations than BNC stations (table 3).

The best ranked model for k_{meth}, which included the percentage of $Fe(III)_{AE}/Fe_T$ as an explanatory variable, does not necessarily indicate the top model for MPP (all sites; fig. 11), as MPP is primarily driven by microbial iron-reduction in the sites sampled for this study. The ambiguous or co-occurring roles of iron reduction or sulfate-reduction as the dominant microbial process that leads to methylation rates in marine estuaries has been noted in recent literature (Merritt and Amirbahman, 2008, 2009; Faganeli and others, 2012). Recent advances in the identification of the Hg(II)-methylation gene cluster (*hgc*AB) have demonstrated that *hgc*AB is abundant in marine sediment overall, and that contaminated marine sediment contains representatives of both iron- and sulfate-reducing bacteria that possess the *hgc*AB gene cluster (Podar and others, 2015).

III.   Release of Mercury Species from Sediment to Water Column

By A.J. Paulson, B. Carter, J.F. DeWild, and R.W. Sheibley

Methylation of inorganic mercury in sediment (see section, "Methylation Potential of Mercury in Sediments") is the first step in the geochemical transformation and transport of MHg to the base of the pelagic food web in the water column. Then MHg must be released into sediment porewater and into the water column.

The maximum porewater FTHg and FMHg concentrations have been detected near the sediment-water interface of most open-water estuarine sediment (Gill and others, 1999; Choe and others, 2004; Muresan and others, 2007; Schäfer and others, 2010). The environmental and geochemical factors controlling porewater FTHg and FMHg in estuarine sediments are unclear. Lower porewater FTHg and FMHg concentrations were measured in winter relative to concentrations measured during warmer periods in San Francisco Bay, California (Choe and others, 2004); Grado Lagoon, Adriatic Sea, Italy (Covelli and others, 2008); New York/New Jersey Harbor (Hammerschmidt and others, 2008); and Thau Lagoon, France (Muresan and others, 2007). However, season does not appear to influence FTHg and FMHg concentrations from sediment in Long Island Sound (Hammerschmidt and Fitzgerald, 2008). In this study, FTHg and FMHg concentrations in porewaters from six stations in Sinclair Inlet (fig. 2) were measured in August 2008, and February, June, and August 2009. Additionally, porewater concentrations of FTHg and FMHg were measured in sediment from three Puget Sound representative bays (fig. 1) in August 2008.

Many estuarine chemists interpret porewater concentrations as in equilibrium with the solid phase of sediment. In this interpretation, porewater concentrations are controlled by solid-phase mercury concentrations and the organic matter of the sediment (Bloom and others, 1999; Hammerschmidt and others, 2008; Hollweg and others, 2009). However, other investigators have reported that porewater FTHg and FMHg concentrations are controlled by other factors associated with the redox condition of the porewaters. Rothenberg and others (2008) concluded FMHg concentrations were related to dissolved porewater ferrous iron (Fe[II]) rather than organic matter or AVS in sediment.

In contrast, Hollweg and others (2009) concluded that methylation and accumulation of FMHg in porewaters was favored when dissolved H_2S concentrations ranged between 3 and 320 µg/L and that FMHg in porewaters is partially controlled by the quality of organic matter in sediment. In this study, DOC was collected from the same composite sample as FTHg and FMHg samples. Additionally, the redox state of the sediment was categorized by the presence of reduced species (ammonia, Fe(II), and H_2S), increased concentrations of filtered total iron and manganese, and the absence of nitrate (an oxidizer).

In order for FTHg and FMHg to be released to the water column, the rate of exchange of porewaters with the overlying water by diffusion or advection must be more rapid than geochemical processes that are removing these species from the aqueous phase at the sediment water interface. Similar to porewater concentrations, seasonal patterns in the flux of FMHg from porewater into the water column have been observed. Gill and others (1999) measured the maximum fluxes in spring and diurnal fluxes of FMHg from sediment in a shallow Texas bay. Hammerschmidt and Fitzgerald (2008) measured higher fluxes in August 2003 than in February 2004. Merritt and Amirbahman (2008) and Benoit and others (2006) suggest that the depth of oxygen penetration into the sediment is a major factor in controlling mercury fluxes from the sediment. In incubation core experiments, Mason and others (2006) detected maximum FMHg concentrations in overlying waters after 20 hours and maximum FTHg concentrations after 60 hours. In this study, fluxes of FMHg and FTHg were calculated from changes in concentrations in water overlying cores incubated in the dark, typically for 1 day.

Of particular note in the literature was the reference to demethylation at the sediment water interface. In one instance, high concentrations of FTHg and FMHg in porewater were measured in White Slough, San Francisco Bay-Delta, California, but the FMHg flux measured by flux chambers was low and the FTHg flux was high (Choe and others, 2004). The authors attributed this discrepancy to either oxygen penetration, lack of bioirrigation, or demethylation at the sediment-water interface.

The reproducibility of replicate flux measurements varied greatly among studies. The relative standard deviation of flux measurements from shipboard incubation studies with triplicate cores collected from New York/New Jersey Harbor generally were less than 10 percent. In contrast, highly variable fluxes, relative standard deviation exceeding 100 percent in some cases, were measured by benthic flux chambers in a Texas bay (Gill and others, 1999), by incubation of cores from Baltimore Harbor, Maryland, and by diffusive

flux calculations from porewater concentrations collected from Thau Lagoon, France. Because previous studies showed large variations of sediment properties in Sinclair Inlet over short distances (Paulson and others, 2012), triplicate core incubation studies were done at each of the six stations for the three seasons in 2009 after the initial pilot study in August 2008, in which only one core incubation experiment could be completed for each site. The STHg of sediment samples collected in greater Sinclair Inlet and OU B Marine in 2009 (fig. 1-1) were within the 95 percent confidence level of the correlation of STHg and total organic carbon of greater Sinclair Inlet sediment collected in 2007 (Paulson and others, 2010).

Porewater and Water Laboratory Methods

Porewater Sampling and Analysis

The collection methods for porewater, core incubation experiments, and tumbling core experiments are shown in figure 4. Details of sample collection, sample processing, and analytical results is available in Huffman and others (2012) along with extensive quality-assurance data and assessments.

Intact and sealed duplicate cores from each station for determination of predominant redox conditions were placed in a nitrogen atmosphere, where the top 2-cm of sediment was packed into polypropylene centrifuge tubes and then centrifuged at 2,000 revolutions per minute (rpm) for 20 minutes. Immediately after opening the centrifuge tube and isolating the unfiltered porewater in a syringe, sulfide and ferrous iron concentrations were measured at various dilutions with laboratory water purged with N_2. After the spectrometric measurements (Huffman and others, 2012) were completed, porewater was filtered through a 0.45 µm, 25-mm, Millex® disk filter into HDPE bottles for analysis of nutrients and Fe_T and Mn.

After centrifugation, the supernatant in the 15 PFA tubes was filtered through multiple quartz fiber filters (QFF) held in a fluorocarbon polymer (FP) filtering tower into a 500-mL FP bottle in the vacuum desiccator in a laminar flow hood. About 60-mL of filtered porewater was transferred to an amber pre-packed bottle for analysis of DOC and total nitrogen by the NRP laboratory in Boulder, Colorado, and the remainder of the porewater in the FP bottle was acidified and analyzed for FTHg and FMHg at the WMRL.

Incubation Experiments

Single cores at all nine stations sampled in August 2008 were incubated at ambient temperatures for Sinclair Inlet in a manner similar to Hammerschmidt and Fitzgerald (2008). Incubation experiments generally were done on three cores (A, B, and C) at each of the six Sinclair Inlet stations during February, June, and August 2009. An initial sample of filtered overlying water (one-half to two-thirds of the volume of overlying water) was collected for analysis of FTHg and FMHg. Filtered, near-bottom water from their respective stations (replacement water) was added in a manner that minimized disturbance of the sediment-water interface. After stirring the overlying water for typically 1 day (2 days in some instances in August 2008), the process of filtering the water overlying the core, adding replacement water, and stirring in the incubator continued daily until the fourth day. All water added and removed, and the sediment held in the apparatus with no overlying water at the end of the experiment were weighed to account for the increase in mass of FTHg and FMHg in the overlying water needed to calculate release rates. Dissolved-oxygen concentrations of overlying water from a subset of cores were always greater than 1 mg/L measured using 0–1 mg/L R-7501 CHEMets (Chemetrics, Calverton, Virginia) and 0–12 mg/L R-7512 CHEMets immediately after the sample was collected on the last day.

Fluxes of FTHg and FMHg were calculated based on the change in concentrations in the test, overlying water during each period between observations over the time of the experiment (*dc/dt* in nanograms per liter per day):

$$Release\ flux = \frac{dc}{dt} * V_{tot}\ /\ A , \qquad (1)$$

where

dc/dt is the rate of change in concentration in the test overlying water, in nanograms per liter per day;

V_{tot} is the total volume, in liters, of water overlying the core; and

A is the cross sectional area of the inside of the core liner (33.3 square centimeters).

The flux rate was calculated between each measurement by substituting:

$$\Delta_c / \Delta_t = (C_d - C_{d-t}) / \Delta_t , \qquad (2)$$

where

C_d is the concentrations of FTHg or FMHg, in nanograms per liter, in the overlying water on the day of the measurement at the end of the elapsed time (Δt); and

C_{d-t} is the calculated concentration of the test overlying water immediately after taking the prior measurement and filling the core liner with replacement water and is calculated by mass balance as

$$C_{d-t} = \left[(C_p * V_p) + (C_r * Vr) \right] / V_{tot} , \qquad (3)$$

where

C_p is the concentrations of FTHg or FMHg, in the water overlying the core liner after withdrawing water for the prior measurement;

C_r is generally the average of the FTHg and FMHg concentrations of near-bottom water collected in the field and the replacement water in PETG bottles stored in the refrigerator for 3 days; and

V_p and V_r are the volume, in liters, of overlying water remaining after the prior measurement, and the volume of replacement water added, respectively, where V_{tot} is equal to the sum of V_p and V_r.

Substituting equation 3 into equation 2, and then substituting $\Delta c/\Delta t$ in equation 2 for dc/dt in equation 1 results in:

$$\text{Release flux} \left[(ng / m^2) / d \right]$$
$$= \left[(C_d * V_{tot}) - [(C_p * V_p) + (C_r * V_r)] \right] / (area * t) . \quad (4)$$

If C_d was less than 0.04 ng/L or ($C_d - C_{d-t}$) was less than 0.07 ng/L (1.66 times the reporting level), the release flux was reported as not detected. Negative release fluxes indicate that FTHg and FMHg were removed from the overlying water by the sediment or the apparatus.

One control-core incubation experiment, with Purelab® Ultra water in a clean core liner sealed at the bottom with a machined polycarbonate plug, was done in August 2008 and four control-core incubation experiments were done in 2009. For the August 2008 control core-incubation experiments, no initial measurements of C_p and C_r were made. After 2 days, FTHg and FMHg concentrations were 0.71 and 0.10 ng/L, respectively. Setting C_d and C_p to zero results in maximum control fluxes of 5 and 32 (ng/m^2)/d for FMHg and FTHg, respectively, for the 2008 control incubation experiments.

For the four control incubation experiments completed in 2009, water in the core liner was measured 15 minutes after filling the apparatus with the Purelab® Ultra water. After 3 days of stirring, FTHg concentrations ranged from 0.12 to 0.71 ng/L. Using the FTHg concentration at 15 minutes for C_p, the median FTHg release flux for the control experiment was 2.5 (ng/cm^2)/d and ranged from 25 to +19 (ng/m^2)/d. The FTHg release flux of the 2009 control experiments for qualification of data was set at the maximum release flux of 19 (ng/m^2)/d. After 3 days, FMHg concentrations ranged from less than 0.04 to 0.15 ng/L. The median FMHg release rate was 2.5 (ng/m^2)/d and ranged from not detectable to 6 (ng/m^2)/d. For consistency with 2008 data, 5 (ng/m^2)/d was used for qualifying FMHg release fluxes. Only in six instances when the value of ($C_d - C_{d-t}$) was 0.07 ng/L or greater, did the calculated FMHg release flux of the 2009 control cores exceed 5 (ng/m^2)/d.

Tumbling Core Experiments

On the evening of sample collection, station water was added to fill the 500-mL FP beakers containing the sediment for the tumbling core experiment. After 15 minutes tumbling end-over-end on a 1-m diameter wheel rotating at 7 revolutions per minute (rpm) for 15 minutes to allow mixing of porewater and added water, the sediment slurry was allowed to settle and the supernatant was filtered through multiple QFF filters. The beaker was filled to the rim with replacement water from the station, sealed, bagged, and secured to the rotating wheel at room temperature. After 2 days, the slurry was allowed to settle and was filtered through multiple QFF filters. Similar to the incubation experiment, the mass of the slurry was recorded at every step, and solids in the beaker during the experiment were dried at 60 °C to calculate the ratio of solids to liquid. The ratio of solids to liquid during the tumbling experiment of Sinclair Inlet samples ranged from 0.09 to 0.2 kg/L, and the ratio was near 1 kg/L for the sandy

CZ sediment. In February, dissolved oxygen concentrations in overlying water from three tumbling cores were greater than 1 mg/L using R-7512 CHEMets immediately after withdrawing a sample for FTHg and FMHg. In August 2009, the overlying water was processed for redox-sensitive species described for the porewater sampling.

The release rate was calculated as follows:

$$\text{Release rate, in nanograms per gram}$$
$$= \left[\left(C_{3d} * V_{tot} \right) - \left(C_{15m} * V_p \right) - \left(C_r * V_r \right) \right] / \left(\text{solids} \right), \quad (5)$$

where C_{15m}, C_{3d}, and C_r are the concentrations of FTHg or FMHg, in nanograms per liter after 15 minutes of mixing, on day three after 2 days of tumbling, and the replacement water, respectively. Volumes (V_{tot}, V_p, and V_r in L), and solids (in grams dry weight) are as indicated for the incubation experiment. During 2 days of tumbling Purelab® Ultra water in a clean beaker without sediment, FTHg increased from 0.22 to 0.26 ng/L.

Laboratory Analyses

The UW Chemical Oceanography Laboratory analyzed nutrients for all porewaters and marine water column samples following the methods of Armstrong and others (1967), and Slawyk and MacIsaac (1972), Bernhardt and Wilhelms (1967). Samples for particulate total carbon and total nitrogen were collected on 0.45-mm pore size, 25-mm diameter, baked glass-fiber filters in a PFA filter holder and measured using U.S. Environmental Protection Agency (EPA) method 440.0 (U.S Environmental Protection Agency, 1997). The filtrate (125 mL) was collected into a baked, glass bottle and acidified with HCl for analysis of DOC (see "Methylmercury Accumulation in the Base of an Estuarine Food Web"). Concentrations of total suspended soldis were measured gravimetrically (6-place balance (precision of 0.001 mg) before and after filtering water through a Nuclepore™ 0.4-μm pore size, 47-mm diameter, polycarbonate filter. After August 2008, total suspended solids measurements were taken from every bottle in which particulate Hg measurements were made.

All marine water samples were filtered through a QFF held in a filtering tower into a 500-mL FP bottles in the vacuum desiccator in a laminar flow hood. The filtrates were collected in FP bottles and acidified, and some QFF filter were placed in FP petri dishes and frozen. Total mercury in filtered water (FTHg) in all other samples was measured by the WMRL using the EPA method 1631, revision E (U.S.

Environmental Protection Agency, 2002) that includes oxidation, purge and trap, desorption, and cold vapor atomic fluorescence spectrometry (CVAFS). Methylmercury in filtered water (FMHg) was first distilled to reduce matrix affects, then ethylated and trapped onto reaction traps (DeWild and others, 2002). The mercury species were thermally desorbed, separated by gas chromatography, and analyzed by CVAFS. The reporting levels for FTHg and FMHg are 0.04 ng/L,

Analysis of total mercury in suspended solids (PTHg) are prepared by room-temperature acid digestion and oxidation with aqua regia followed by overnight heating (50 °C) in a 5-percent (w/v) bromine monochloride solution to ensure complete oxidation (Olund and others, 2004). The solution was analyzed by CVAFS. The method detection limit of PTHg is 0.059 ng of mercury per filter. Analysis of methylmercury in suspended solids (PMHg) was prepared by extraction with potassium bromide, copper sulfate, and methylene chloride (DeWild and others, 2004). The methylmercury was solvent extracted with methylene chloride and back extracted in water by evaporation of the methylene chloride. The methylmercury was then analyzed in a manner similar to the ethylation, chromatographic separation, and analysis by CVAFS. The reporting level for PTHg is 0.01 ng of mercury per filter. More detailed methods and quality assurance measures are reported in Huffman and others (2012).

Mercury Concentrations in Porewater

The biochemical accumulation of FTHg and FMHg in the porewater of sediment is necessary to release FTHg and FMHg to the water column of Sinclair Inlet. FTHg and FMHg are released to the water column when the porewater is transported out of the sediment by physical processes. In August 2008 (table 5, fig. 12), high FTHg concentrations (35.9 –59.8 ng/L) and high FMHg concentrations (20–28 ng/L) were measured in porewater from the two stations with highly reducing sediment (SI-PO and LB) and from one station with moderately reducing sediment (SI-IN). For the other two representative bays (HH and BI) and the other greater Sinclair Inlet station (SI-OUT) that were categorized as moderately reducing, FTHg concentrations ranged from 3.19 to 10.8 ng/L and FMHg concentrations ranged from 0.08 to 1.30 ng/L. For the three OU B Marine stations that were characterized as reducing, FTHg concentrations in porewater ranged from 2.23 to 6.21 ng/L and FMHg concentrations ranged from 0.09 to 0.36 ng/L.

Table 5.   Predominant redox conditions, dissolved organic carbon and mercury concentrations in porewater, and releases during core incubation and tumbling experiments from sediment collected from Sinclair Inlet, Kitsap County, Washington, 2008 and 2009.

[**Station:** BI, Budd Inlet; BNC, Bremerton naval complex; CZ, convergence zone; HH, Holmes Harbor; LB, Liberty Bay; OU B Marine, Operable Unit B Marine; SI-IN, Sinclair Inlet-inner; SI-OUT, Sinclair Inlet-outer; SI-PO, Sinclair Inlet-Port Orchard; *p* value, probability of difference between representative bays and Sinclair Inlet including OU B Marine. **Area:** SI, Sinclair Inlet; RB, representative bay. **Redox:** HR, highly reducing; MR, moderately reducing; R, reducing; WR, weakly reducing. **N:** Number of core incubation experiments. **Abbreviations:** mg/L, milligram per liter; ng/L, nanogram per liter; mg/kg, milligram per kilogram; [ng/m^2]/d, nanogram per square meter per day; NA, not analyzed; nd, not detected]

Station	Area	Redox	Porewater concentrations			N	Median flux from sediment ([ng/m^2]/d)		Release during tumbling (mg/kg)	
			Dissolved organic carbon (mg/L)	Filtered total mercury (ng/L)	Filtered methylmercury (ng/L)		Filtered total mercury	Filtered methylmercury	Filtered total mercury	Filtered methylmercury
August 2008										
SI-OUT	SI	MR	7.6	9.97	0.08	1	nd	nd	NA	NA
SI-PO	SI	HR	4.0	35.9	20	1	491	353	NA	NA
SI-IN	SI	MR	8.7	59.8	28	1	338	5	NA	NA
BNC-52	OUB Marine	R	3.8	6.21	0.09	1	nd	nd	NA	NA
BNC-39	OUB Marine	R	4.3	2.86	0.2	1	nd	nd	NA	NA
BNC-71	OUB Marine	R	3.8	2.23	0.36	1	E 32	nd	NA	NA
HH	RB	MR	4.5	10.8	1.30	1	48	nd	NA	NA
LB	RB	HR	8.9	58.1	26	1	NA	329	NA	NA
BI	RB	MR	4.9	3.19	0.52	1	108	nd	NA	NA
p value			0.3	0.41	0.45					
February 2009										
SI-OUT	SI	R	2.5	8.62	0.04	3	67	nd	0.007	0.000
SI-PO	SI	WR	3.3	6.18	0.07	3	22	nd	0.011	0.000
SI-IN	SI	R	2.5	3.1	0.1	3	nd	nd	0.004	0.001
BNC-39	OU B Marine	MR	4.1	16.9	0.14	3	41	nd	NA	0.001
BNC-60	OU B Marine	R	2.8	2.72	0.24	3	73	nd	NA	0.000
BNC-71	OU B Marine	MR	3.1	6.99	0.09	3	nd	nd	0.008	0.005
CZ	SI	NA	NA	NA	NA	NA	NA	NA	0.070	0.001
June 2009										
SI-OUT	SI	R	5.5	7.87	0.58	3	nd	nd	-0.0053	0.0026
SI-PO	SI	R	7.5	28.9	18	2	75	128	NA	NA
SI-IN	SI	R	6.6	47.3	20	3	1,462	1,079	1.635	0.929
BNC-39	OUB Marine	MR	4.9	4.47	0.63	3	25	6	0.143	0.098
BNC-60	OUB Marine	R	7.8	41.7	15	3	333	372	0.563	0.269
BNC-71	OUB Marine	MR	4.6	22.7	1.5	3	100	8	0.454	0.349
CZ	SI	NA	NA	NA	NA		NA	NA	0.093	0.007
August 2009										
SI-OUT	SI	WR	3.6	10.9	0.1		94	nd	-0.001	0.002
SI-PO	SI	WR	5.4	4.56	2.8		247	264	0.60	0.44
SI-IN	SI	WR	2.7	2.95	0.36		200	212	0.07	0.06
BNC-39	OUB Marine	MR	4.5	5.89	0.46		nd	nd	0.02	0.01
BNC-60	OUB Marine	R	5.0	5.07	1.1		26	nd	0.21	0.12
BNC-71	OUB Marine	MR	5.6	16.2	1.4		nd	nd	0.43	0.15
CZ	SI	NA	NA	NA	NA		NA	NA	0.02	0.00
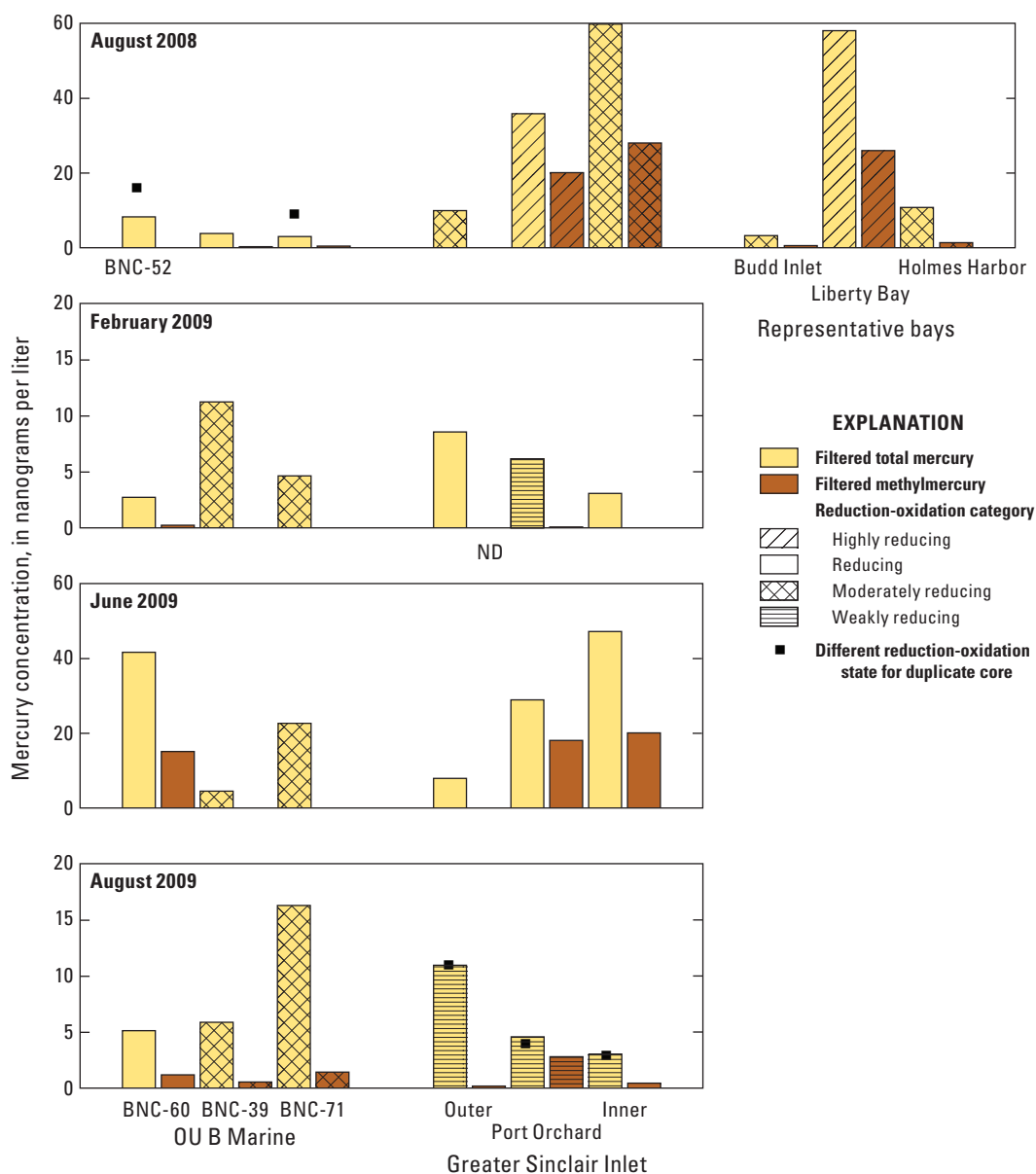
Figure 12.   Filtered total mercury and filtered methylmercury concentrations in porewater for four seasonal sampling periods, Sinclair Inlet, Kitsap County, Washington, between August 2008 and August 2009. Representative bays Holmes Harbor (HH), Liberty Bay (LB) and Budd Inlet (BI) stations were sampled only in August 2009. Only one methylmercury value was not detectable. Note changes in the range of mercury concentrations (y-axis) among seasons.

In February 2009, FMHg concentrations in porewater were low in OU B Marine (0.09–0.14 ng/L) and greater Sinclair Inlet (<0.04–0.1 ng/L) stations. No clear trend between FTHg concentrations and redox state was observed during February 2009. Considerable overlap of FTHg concentrations was noted across redox states for all stations (reducing [2.72–8.62 ng/L], moderately reducing [6.99–16.9 ng/L], and weakly reducing [6.18 ng/L]). The highest FTHg concentrations (28.9–47.3 ng/L) and FMHg concentrations (15–20 ng/L) in porewater measured in June 2009 were collected from stations with reducing sediment (the most reducing redox condition for this sampling period). Similar to August 2008, a high FTHg concentration (22.7 ng/L) was measured in porewater in June 2009 from a station with moderately reducing sediment (BNC-71). Unlike porewater from SI-IN collected in August 2008, low FMHg (1.5 ng/L) was measured in moderately reducing sediment from BNC-71 in June 2009. Reducing porewater from SI-OUT contained lower concentrations of FTHg (7.87 ng/L) and FMHg (0.58 ng/L).

Sediment collected in August 2009 was less reducing than that collected at the same stations in August 2008. The FMHg concentrations (average 1.0 ng/L; range 0.1 to 2.8 ng/L) in porewater collected in August 2009 was lower than corresponding concentrations (average 9.7 ng/L; range 0.08 to 28 ng/L) collected in August 2008 from five common stations. Unlike the results from August 2008, high FTHg concentrations were measured in porewater at stations with moderately reducing sediment (5.89–16.2 ng/L) and weakly reducing sediment (10.9 ng/L).

Redox State of Porewater

The method for assessing the predominant redox conditions of the sediments was based on the presence or absence of electron acceptors (nitrate) or the presence of reduced byproducts (H_2S; and dissolved ammonia, Fe, and Mn). The predominant redox state of a station is not an exact measurement because of vertical and horizontal heterogeneity. The top 2 cm of sediment likely contains more than one of the redox states, so species (for example, nitrate and sulfide) that are not chemically stable in the presence of each other may nevertheless co-exist at low concentrations during the short time that the 2-cm interval of porewater is extracted. Therefore, the identified predominant redox state at a station at a particular time may not be entirely consistent with the presence or absence of all chemical species. If duplicate measurements were available for a sampling station, they were included in all statistics that involved categorical summaries such as comparison between seasons or between location groups to fully represent the site-specific variability.

Because concentrations of nitrate and sulfide were critical to categorizing the predominant redox state of porewater, threshold values of nitrate and sulfide were established for each redox category. During August 2008, and February and June 2009, nitrate concentrations were relatively low and less than three times the interquartile range greater than the means for the sampling event (0.018, 0.007, and 0.009 mg/L as nitrogen, respectively). During August 2009, one of two replicate cores from each of the three greater Sinclair Inlet stations contained substantially more nitrate (0.107 to 0.365 µg/L as N). The threshold nitrate concentration for the other nine samples was 0.105 mg/L as nitrogen. When the spectrometric reading for sulfide concentrations was adjusted for the dilution factor of the sample, a natural break in the data was measured at 0.26 mg/L (8 micromolar) and was used as threshold for the presence of sulfide. This sulfide concentration is similar to sulfide concentrations critical to methylation processes (Hollweg and others, 2009).

Detectable concentrations of dissolved Fe and Mn also were measured in most porewaters, but provided little information on identifying predominant redox state. Dissolved Mn was detected in all porewater samples at concentrations ranging from the reporting limit (20 µg/L) to 2,840 µg/L. After adjusting for dilution, reporting levels for dissolved Fe generally were 400 or 800 µg/L, which were too high to determine the onset of ferric iron reduction.

Four sediment redox states were established. Sediment cores containing nitrate, dissolved Mn but no sulfide greater than the threshold were classified as "weakly reducing." For the "Moderately reducing" category, cores did not contain nitrate, did contain dissolved Mn, and did not contain sulfide greater than threshold values. "Reducing" cores did not contain nitrate, did contain dissolved Mn and sulfide greater than threshold values. When sulfide concentrations generated by sulfate reduction become sufficiently high, Fe and Mn sulfides begin to precipitate, leading to decreased Fe and Mn concentrations. "Highly reducing" cores contain (1) high levels of sulfide (greater than 2.4 mg/L overage range of the measurement at a dilution of 1:4), (2) nitrate and Fe concentrations less than the reporting level, and (3) Mn concentrations less than or equal to 100 µg/L. For 19 of 24 duplicate cores, the redox state of the duplicate cores was the same (Huffman and others, 2012). If the redox state of the duplicate cores differed, the redox state of the station was set at the more reducing state.

In August 2008, highly reducing sediments were detected only at stations SI-PO and LB (table 5). Two representative bays (HH and BI) and two greater Sinclair Inlet stations (-IN and -OUT) were classified as moderately reducing. The three BNC stations were classified as reducing due to sulfide concentrations greater than the threshold of 0.26 mg/L.

During 2009, the redox state of the greater Sinclair Inlet stations changed throughout the year. In February, the predominant redox state of the SI-PO station was "weakly reducing," whereas it was "reducing" at stations SI-IN and SI-OUT. In June, the predominant redox state at the three greater Sinclair stations was reducing, and weakly reducing in August 2009. The predominant redox state at the OU B Marine stations remained unchanged during 2009; which was reducing at BNC-60 and moderately reducing at BNC-39 and BNC-71.

Fluxes of Total Mercury and Methylmercury from Sediment

Fluxes of FTHg and FMHg occur through the physical and biological processes that transport porewaters across the sediment-water interface into the water column. The stirring conditions of the core incubation experiments for this study reflect the low tidal-energy conditions often observed in Sinclair Inlet (Gartner and others, 1998). For each season, there were similarities in the trends of the release fluxes of FTHg and FMHg with the trends in porewater concentrations; however, there also were notable exception to the trends.

Similar to the high FTHg and FMHg porewater concentrations in August 2008, high fluxes of FTHg in August 2008 (table 5) were measured from cores collected from the two stations with highly reducing sediment, SI-PO and LB. Flux of FMHg in August. Fluxes of FMHg in August 2008 could not be determined due to analytical difficulty. Similarly, a relatively high flux of FTHg was measured from moderately reducing sediment from SI-IN that also contained high porewater concentration of FTHg. However, the flux of FMHg from the SI-IN core was at the detection limit of the flux measurement (5 [ng/m^2]/d), even though FMHg porewater concentrations (28 ng/L) from SI-IN were the highest measured during the study. Fluxes of FMHg were less than the detection limit for the other six cores. The FTHg fluxes of 48 and 108 (ng/m^2)/d were measured from the two other representative bays (HH and BI, respectively).

For the six SI stations sampled in February, June, and August 2009, the fluxes of FTHg and FMHg from triplicate cores were measured over 3 days. The variability of FMHg fluxes among the triplicate cores from BNC-60 collected in June 2009 were typical of the daily fluxes results detected among other triplicate cores. The FMHg fluxes were significantly different between the three cores during the first day of the experiment (day 0–1; fig. 13). Fluxes near the end of the experiment for cores A and C were less than fluxes on the first day, but the FMHg flux of core B was negative on the second day. The FTHg and FMHg fluxes generally decreased as experiments progressed in the laboratory. Because the flux measured between sample collection and 1 day later probably best reflects natural conditions, the first acceptable measurement from each replicate core is described and presented in table 1-1.

Similar to the low porewater concentration of FMHg, median FMHg fluxes for all six stations in February 2009 (median concentrations of the triplicate cores are presented table 5) were less than the detection limit. Only 1 of 16 FMHg flux measurements was detectable (6.4 [ng/m^2]/d) at rates only slightly greater than the fluxes from the control cores (table 1-1). Median fluxes of FTHg from all stations were low (<75 [ng/m^2]/d) and dependent on redox conditions.

In June 2009, high fluxes of FTHg (table 1-1) were released from reducing sediment from two of three cores from SI-IN (median 1,462 [ng/m^2]/d; table 5) and from two of three cores from BNC-60 (median 333 [ng/m^2]/d). Lower fluxes of FTHg were measured from reducing sediment from SI-PO (median 75 [ng/m^2]/d) and moderately reducing sediment from BNC-71 (median 100 [ng/m^2]/d). Fluxes of FMHg greater than 100 (ng/m^2)/d were measured from five of the eight cores from Sinclair Inlet stations with reducing sediment (SI-PO, SI-IN, BNC-60 in table 1-1).

The median flux of FTHg from weakly reducing sediment collected in August 2009 from greater Sinclair Inlet ranged from 94 to 247 (ng/m^2)/d (table 5). The fluxes of FMHg from SI-PO and SI-IN were similar to FTHg, but no FMHg was released from SI-OUT sediment. The median flux of FTHg from sediment from OU B Marine stations was less than 30 (ng/m^2)/d and a detectable flux of FMHg was measured from only one of nine cores from OU B Marine (table 1-1). Overall, the measured fluxes were highly variable.
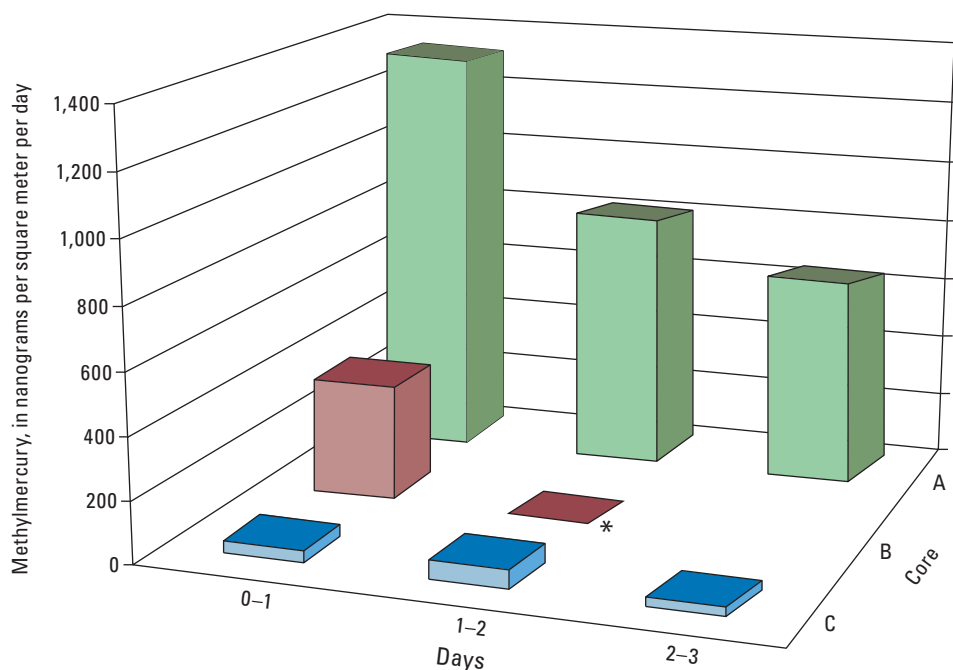
Figure 13. Daily fluxes of filtered methylmercury from triplicate experiments during 3-day core-incubation experiments from Bremerton naval complex (station BNC-60), Sinclair Inlet, Kitsap County, Washington, June 2009. (*Indicates a negative value, -200 [ng/m^2]/d, for this core and time point.)

Release of Total and Methylmercury from Sediment During Tumbling

High-energy windstorms occasionally occur in the Sinclair Inlet area. To assess release of mercury when sediment is suspended into the water column during such high energy storms, water from each station was added to the top 2-cm of sediment and the slurry was mixed by slow end-over-end tumbling for 2 days. The supernatant of the slurry at the beginning of the experiment represents the weighted average of sediment porewater and site water; FTHg and FMHg concentrations generally were less than 9 and 1 ng/L, respectively. Decomposition of organic matter in the slurry in the sealed beaker could change the redox conditions over the 2-day experiment. In August 2009, concentrations of redox-sensitive species were measured in the slurry at the end of the experiment. Except for the slurry containing SI-PO sediment where significant concentrations of sulfide were measured, the slurries were categorized as moderately reducing.

Little THg or MHg (less than 0.07 ng/g) was released from sediment collected during February 2009 (table 5). Sediment collected in June 2009 released the most FTHg and FMHg after tumbling, with four of the six sediment slurries releasing 0.1 ng/g or more (table 5). The slurry of sediment from SI-IN tumbled for 2 days release of 1.635 ng/g of FTHg. In August 2009, greater than 0.1 ng/g of THg was released from three of the seven samples. Little THg or MHg was released from sediment from the open water stations CZ and SI-OUT. When more than 0.1 ng/g THg was released, the ratio of MHg release to THg release was generally greater than 50 percent.

Mercury Concentrations in Water Column

The concentrations of mercury in the water column reflect a baseline marine concentration with inputs from sediment and near-surface terrestrial and atmospheric sources that are moderated by biological processes and turbulent mixing by

tidal forces (Paulson and others, 2012). The rates of inputs from sedimentary and terrestrial sources and biotransformation processes in the water column were manifested if their relative rates were much faster than physical processes that tend to mask these sources and processes. Examining the differences between near-surface and near-bottom concentrations provides insight into the relative rates of physical mixing and other factors. When sufficient ancillary data are available, the probable causes of exceptionally high concentrations (outliers) of any of the four mercury species (FTHg, FMHg, PTHg, and PMHg) were examined. Although some seasonal differences in surface-water concentrations are apparent in graphs in this section, seasonal biological processes that affect Hg species are described in detail in section, "Methylmercury Accumulation in the Base of an Estuarine Food Web."

## Water Column Mercury in Sinclair Inlet Compared to Representative Bays

In August 2008, there was no significant difference (*p* values in table 1-2) in FTHg concentration in both near-surface or near-bottom waters between Sinclair Inlet and the representative bays (fig. 14). The range of the FTHg concentrations in representative bays (0.13–0.42 ng/L) encompassed the range for Sinclair Inlet (about 0.25–0.40 ng/L) for the August 2008 sampling period. As expected for the representative bays, Holmes Harbor had the lowest FTHg concentrations (0.13–0.16 ng/L), whereas FTHg concentrations in Budd Inlet were slightly higher than Holmes Harbor (0.20–0.24 ng/L) and FTHg concentrations in Liberty Bay were about 0.42 ng/L. The ranges of FMHg concentrations in the representative bays were also similar to those in Sinclair Inlet for near-surface and near-bottom samples (fig. 15).

Concentrations of THg of suspended solids (table 1-3) from the representative bays ranged from 0.017 to 0.270 µg/g (fig. 16). Concentrations of THg of suspended solids in Sinclair Inlet in August 2008 (0.066–0.392 µg/g for near-surface solids and from 0.122 to 1.025 µg/g for near-bottom solids from Paulson and others, 2012) were not significantly different from those of representative bays (*p*= 0.25 and 0.14, respectively, table 1-2). No significant differences (*p* = 0.29 and 0.55, respectively; table 1-2) were noted between concentrations of MHg of suspended solids (table 4) between the representative bays and Sinclair Inlet for near-surface and near-bottom waters (fig. 17), respectively.

Importance of Sedimentary Sources of Mercury Species

Statistical examination of differences between near-surface and near-bottom waters provides an overall perspective on the general importance of sedimentary sources relative to near-surface terrestrial and atmospheric sources. For each of the four seasonal sampling periods, differences between near-surface and near-bottom concentrations in Sinclair Inlet were examined for each parameter. This seasonal grouping by water column layers emphasizes the overall stratification of the water column in Sinclair Inlet rather than differences between layers at a specific site. This grouping is appropriate because of the large tidal excursion in Sinclair Inlet (kilometers in scale) and the difference in time of collection between the near-surface and near-bottom samples (several hours in some instances).

Despite significant differences (table 1-2) in some biogeochemical constituents (that is, filtered ammonia [fig. 1-2*A*], filtered nitrate plus nitrite, [fig. 1-2*C*], filtered orthophosphate [fig. 1-2*B*], and filtered silicate [fig. 1-2*D*]), few significant differences in mercury concentrations between near-surface and near-bottom concentrations were noted in Sinclair Inlet (figs. 14–17, table 1-2). Only 3 of 12 seasonal comparisons of FTHg, PTHg and THg of suspended solids (mass of particulate total mercury per mass of suspended solids) between near-surface and near-bottom concentrations were significantly different. The FTHg concentrations in the near-surface layer of Sinclair Inlet in August 2009 were significantly higher than concentrations in the near-bottom layer. In contrast, both concentrations of PTHg and THg of solids (particulate total mercury of a mass per volume of water and per mass of suspended solids, respectively) in the near-bottom layer during June 2009 were significantly higher than concentrations in the near-surface layer. Both these instances of significant vertical differences were associated with water column layers containing exceptionally high concentrations. There were no significant differences (table 2) in concentrations of FMHg (fig. 15) and of MHg of suspended solids (fig. 17) between near-surface and near-bottom layers. Only the suspended solid concentration of MHg (in nanograms per liter) for the August 2009 sampling period was significantly different (*p* = 0.04 in table 2).
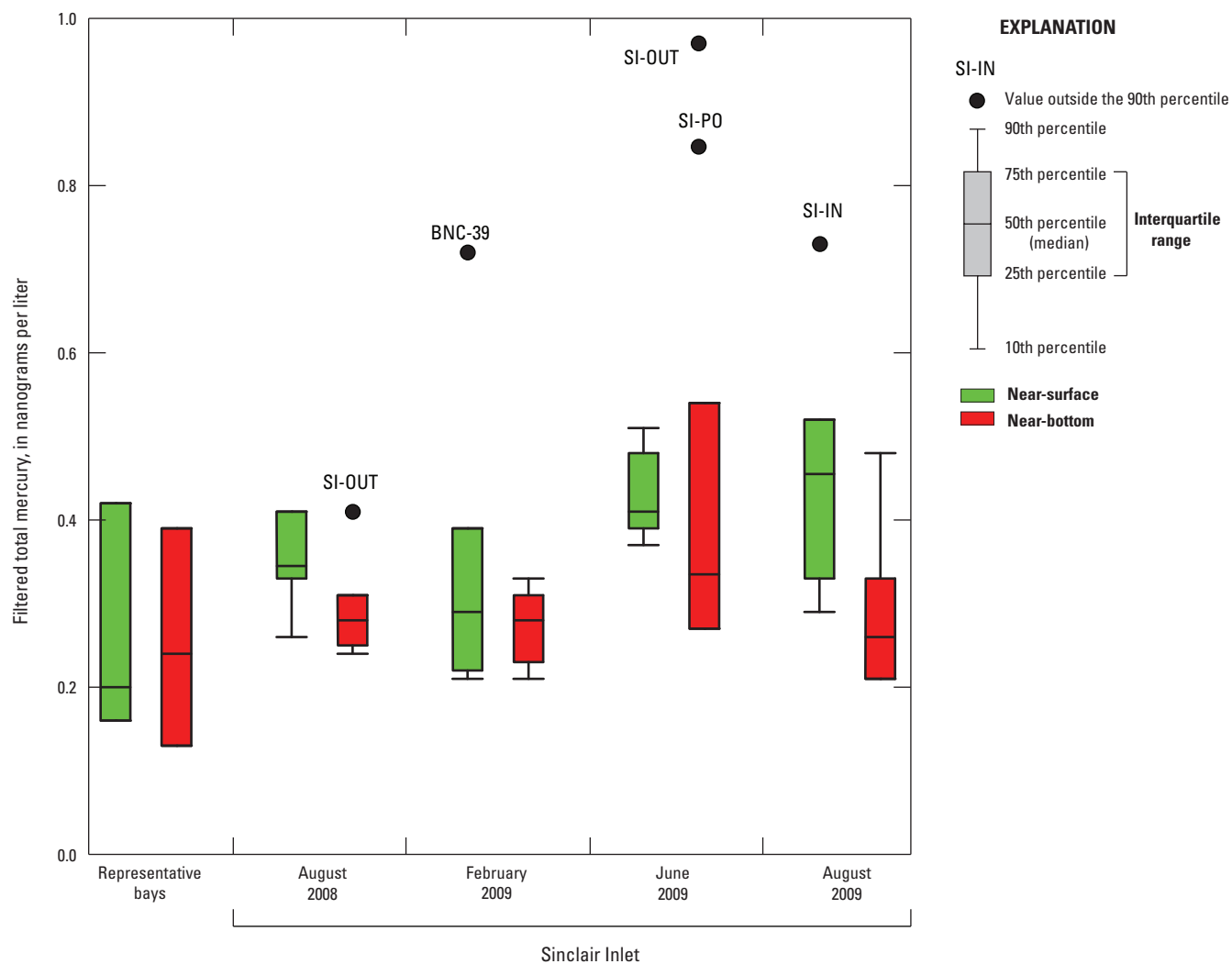
Figure 14.   Filtered total mercury in near-surface and near-bottom water in representative bays (Holmes Harbor, Liberty Bay, and Budd Inlet) and Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.
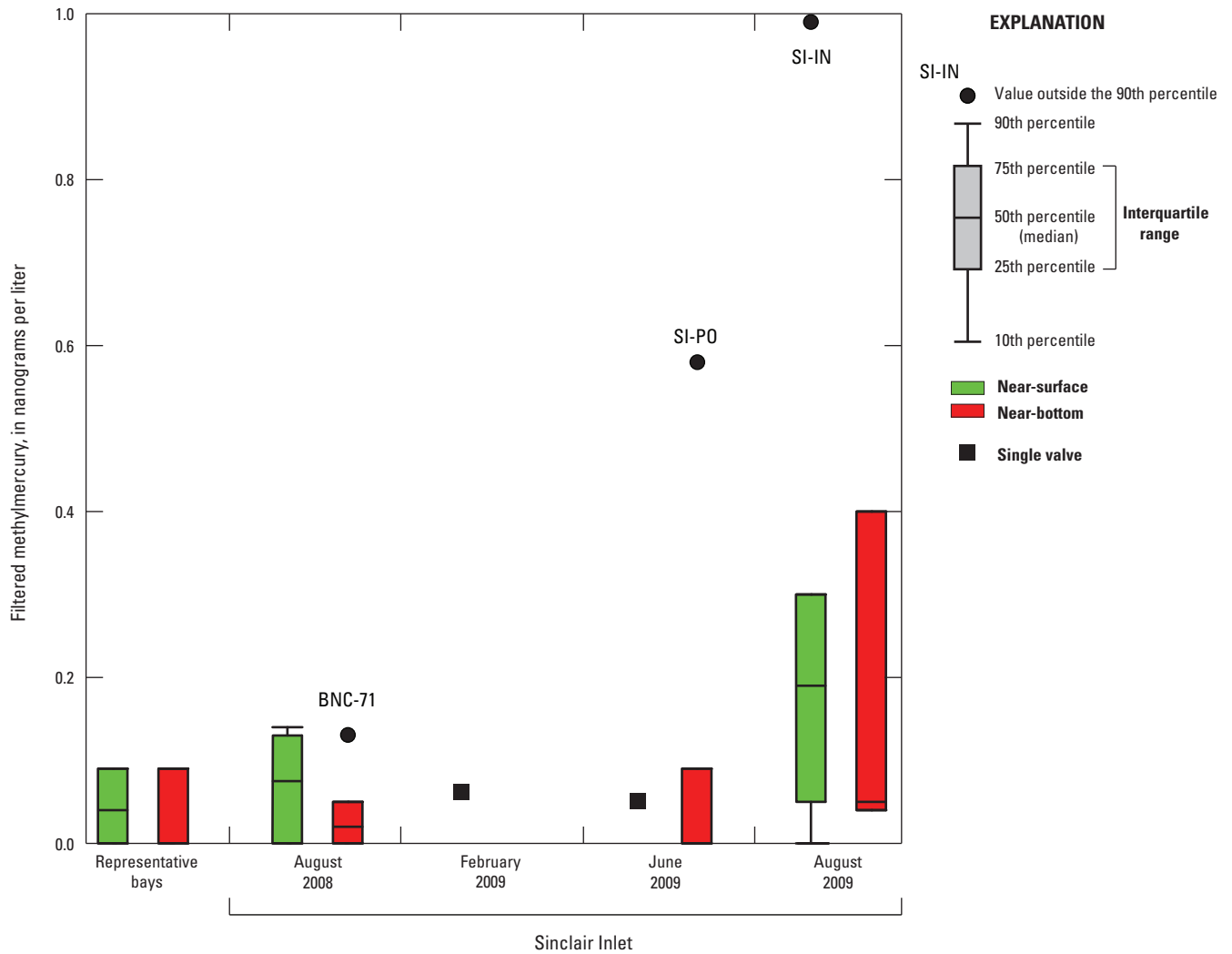
Figure 15.   Filtered methylmercury in near-surface and near-bottom water in representative bays (Holmes Harbor, Liberty Bay, and Budd Inlet) and Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009. (In February and June 2009, one near-surface filtered methylmercury concentration slightly above the detection limit were measured and are represented as unlabeled squares.)
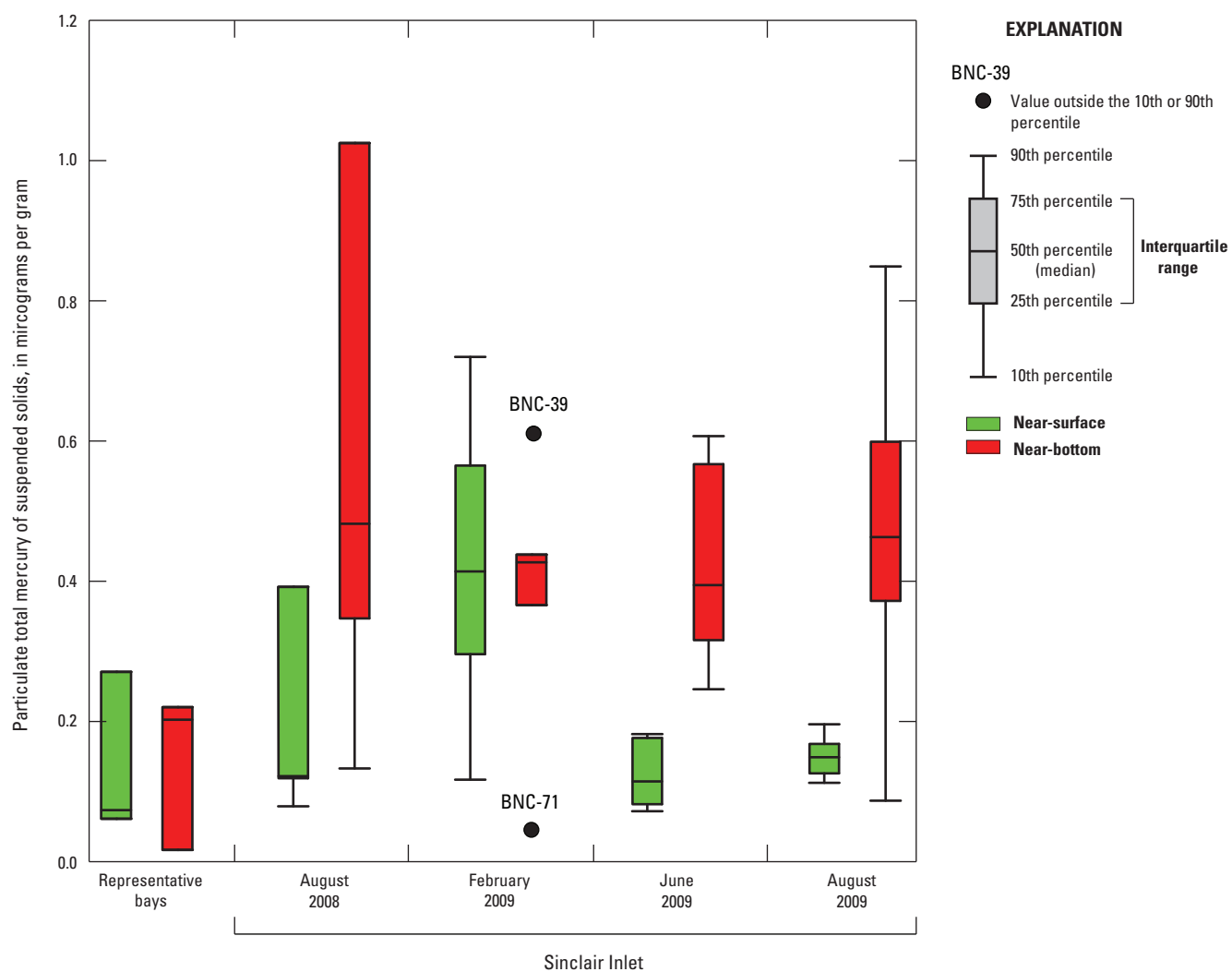
Figure 16.   Total mercury of suspended solids in near-surface and near-bottom water in representative bays (Holmes Harbor, Liberty Bay, and Budd Inlet) and Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

Figure 17.   Methylmercury of suspended solids in near-surface and near-bottom water in representative bays (Holmes Harbor, Liberty Bay, and Budd Inlet) and Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

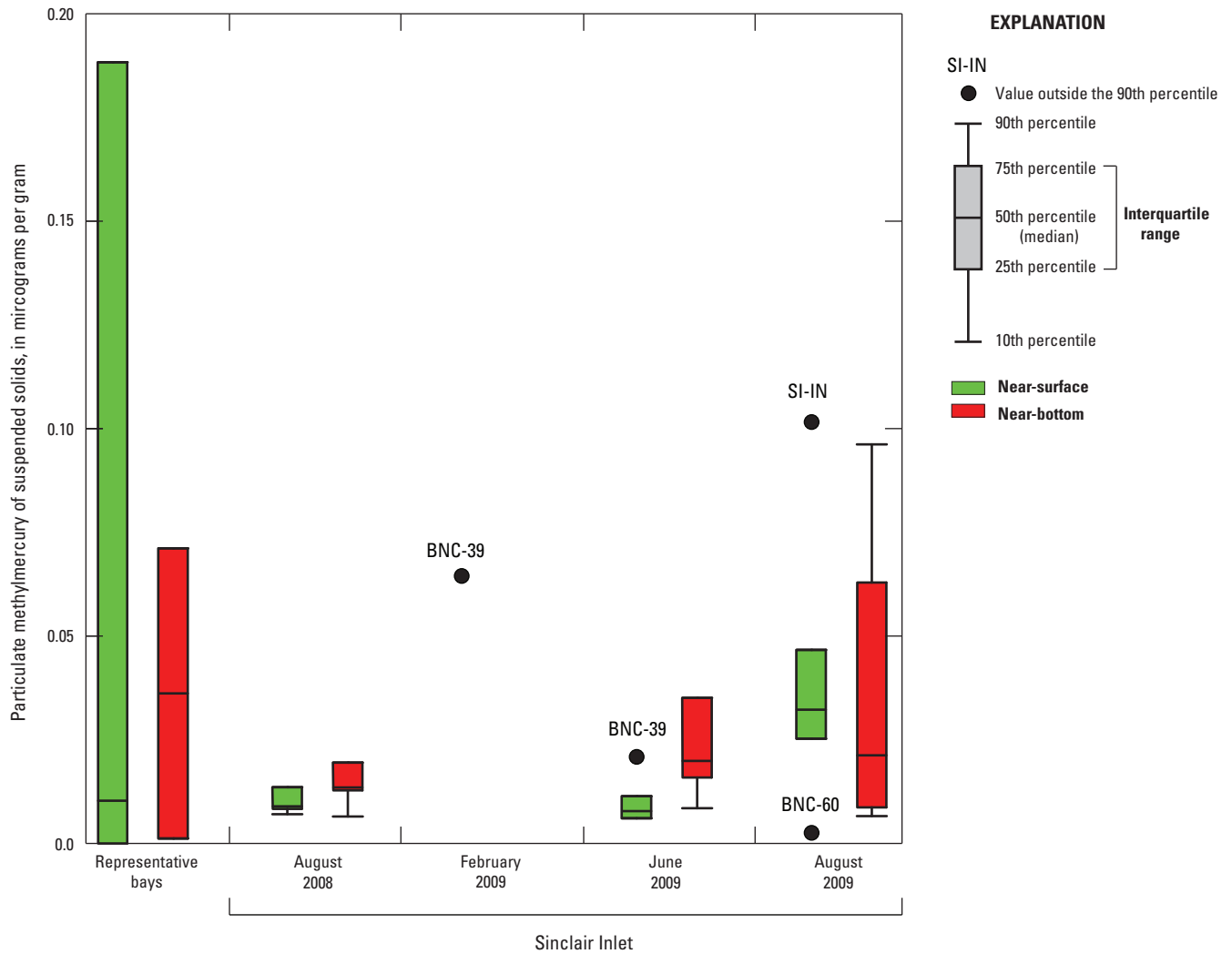Isolated instances of exceptionally high or low concentrations of a mercury species at a specific site (table 1-5) provide insights into local biogeochemical processes affecting a mercury source. Increased concentrations of FTHg and FMHg in surface water were measured at station SI-IN in August 2009 (figs. 14–15). Although the highest orthophosphate concentration of this seasonal dataset (0.17 mg/L as phosphate) was associated with near-surface layer sample in August 2009 SI-IN (fig. 1-2*B*), the silicate concentration was not elevated (fig. 1-2*D*) and nitrate plus nitrite essentially was depleted (0.002 mg/L; fig. 1-2*C*). These observations indicate that the FTHg and FMHg did not originate from a sedimentary source. Exceptionally high concentrations of MHg of suspended solids (0.083 and 0.119 µg/g in table 1-4), total suspended solids (average of 13.1 in table 1-4), an atomic ratio of carbon to nitrogen of the suspended solids (9.33 in table 1-4) and chlorophyll *a* (150 µg/L in Huffman and others, 2012) were measured at SI-IN during August 2009. Thus, the phytoplankton bloom conditions in August 2009 accumulated carbon, nitrogen, and phosphorus, THg, and MHg in the particulate phase. As the phytoplankton died, they released these species into aqueous or colloidal phases. Nitrate is limiting and is reused to produce new phytoplankton, which leaves an accumulation of filterable orthophosphate. Colloidal THg and MHg from the phytoplankton breakdown debris that can pass through the pores of the quartz-fiber filter probably are responsible for the elevated FTHg and FMHg concentrations during bloom conditions at SI-IN during August 2009.

The second highest FTHg concentration of the seasonal Sinclair Inlet set (0.84 ng/L) was measured in near-bottom waters of SI-PO in June 2009 and was associated with an exceptionally high FMHg concentration (0.58 ng/L). Ammonia, orthophosphate, silicate, and manganese are species that are released to the water column of Puget Sound as a result of diagenetic processes occurring in sediment (Paulson and others, 1988; Brandes and Devol, 1997; Kuwabara and others, 2009). Near-bottom waters collected from station SI-PO in June 2009 contained high concentrations of filtered ammonia, orthophosphate, and silicate (table 1-2 and fig. 1-2), and the highest concentrations of manganese detected during the study (fig. 1-3). These elevated near-bottom concentrations of diagenetic species and FMHg suggest enhanced release of porewaters to the water column. The most likely conditions are (1) submarine discharge of groundwater that advects porewater with high concentrations of nutrients, manganese, FTHg, and FMHg deeper in the sediment column to the sediment-water interface and into the water column, or (2) enhanced exchange of porewater and near-bottom water by biological or physical processes.

Data from a CTD cast at SI-PO collected in early May as part of a special study on THg concentrations of solids show evidence of submarine groundwater discharge (fig. 1-4). In the bottom 0.3 m of the water column, salinity decreases from 29.3 to 28.7 PSU. The large decrease in dissolved-oxygen concentrations near the sediment-water interface indicates the upward movement of oxygen-depleted porewaters into the water column. The consistency of the turbidity values (not shown) in the bottom 0.3 m indicates that the CTD sensors had not entered the nepheloid layer above the sediment-water interface. Activity of invertebrates in the sediment can irrigate sediment, and thus enhance water exchange across the sediment-water interface. The low fluxes of FTHg from the incubation experiments with SI-PO cores collected during June 2009 argues against enhanced biological mixing.

Correlations Between Porewater, Fluxes, and Water Column Constituents

Examination of correlations between FTHg, FMHg and DOC concentrations is procedurally consistent because these analyses were done on aliquots from the same bottle of porewater composited from the supernatant from the 15 tubes of centrifuge sediment from multiple box cores collected at each station. The statistical correlation between FTHg and DOC in 27 porewater samples collected between August 2008 and August 2009 is highly influenced by samples with reducing and highly reducing sediment (fig. 18). The non-parametric correlation coefficient (r) (table 6) between FTHg and DOC in reducing and highly reducing sediment (sulfide concentrations greater than 0.26 mg/L, 11 samples) is greater than the r for all 27 samples. The non-parametric correlation of between FTHg and DOC in weakly and moderately reducing sediment (sulfide concentrations less than 0.26 mg/L, 14 samples) was not significant for both non-parametric tests.

The FMHg concentrations were correlated with DOC (fig. 19) regardless of redox state of the sediments. However, the correlation coefficients generally were higher for reducing and highly reducing sediment than for weakly and moderately reducing sediment. Similar to FTHg, the percentage of FTHg in the FMHg form was not significant for weakly and moderately reducing sediment.

Non-parametric multi-comparison Tukey tests were done to examine the correlations between FTHg, FMHg and DOC and respective redox states (table 7) because the redox state is a categorical variable; samples for redox constituents were collected from different sediment cores than samples collected for mercury and DOC. The only significant difference in FTHg concentrations among redox state was the difference
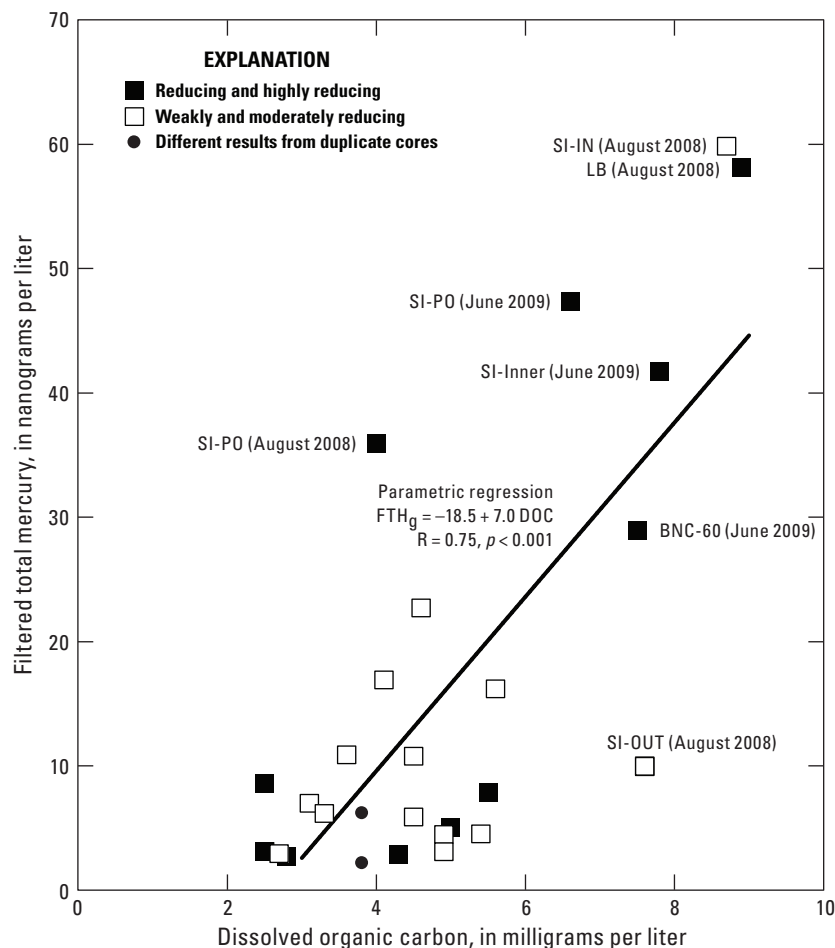
Figure 18.   Filtered total mercury concentrations in porewaters compared to dissolved organic carbon concentrations grouped by two redox conditions, Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009. Sample with filtered total mercury concentrations greater than 25 ng/L and samples separated from the regression line are labeled.

between moderately and weakly reducing sediment (Tukey multi-comparison category B) and highly reducing sediment (Tukey multi-comparison category A). The absence of other significant differences was a result of the large range in FTHg concentrations for the 11 samples of reducing sediment (2.23–47.3 ng/g) and 10 samples of moderately reducing sediment (3.19–59.8 ng/L). The FMHg concentrations in the two highly reducing sediment samples were significantly greater than samples of other redox states. Similar to FTHg, the variation in FMHg concentrations in reducing sediment (<0.04–20 ng/g) and in moderately reducing sediment (0.08–28 ng/L) resulted in no significant differences between these two redox categories. There were no differences in percentage of mercury in the MHg form or DOC among redox states.

Table 6.   Non-parametric regression and parametric correlation statistics of filtered total mercury, filtered methylmercury, and percentage of methylmercury in porewaters compared to dissolved organic carbon categorized by porewater sulfide concentration, Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

[Bold values indicate a statistically significant difference. **Abbreviations:** BNC, Bremerton naval complex; mg/L, milligram per liter; ng/mg, nanogram per milligram; p, probability; r; correlation coefficient; >, greater than; < , less than; –, parametric regression data are not given for data sets in which both non-parametric tests indicate the correlation is not significant]

Sulfide grouping	Number of samples	Non-parametric				Parametric			
		Spearman Rho		Kendall Tau b		Pearson			
		r	p	r	p	r	p	Intercept	Slope (ng/mg)
Filtered total mercury									
All samples	[1]27	**0.58**	**0.002**	**0.42**	**0.003**	0.75	**< 0.0001**	-18.5	7.0
Sulfide > 0.26 mg/L[2]	11	**0.69**	**0.019**	**0.55**	**0.019**	0.79	**0.003**	-16.2	7.3
Sulfide < 0.26 mg/L[3]	14	0.28	0.329	0.21	0.297	0.67	**0.008**	–	–
Filtered methylmercury									
All samples	27	**0.71**	**< 0.0001**	**0.58**	**< 0.0001**	0.77	**< 0.0001**	-12.0	3.5
Sulfide > 0.26 mg/L	11	**0.76**	**0.006**	**0.61**	**0.010**	0.76	**0.007**	-9.2	3.5
Sulfide < 0.26 mg/L	14	**0.59**	**0.027**	**0.50**	**0.014**	0.69	0.006	12.1	3.1
Methylmercury (percent)									
All samples	27	**0.58**	**0.003**	**0.42**	**0.003**	0.60	0.008	-14.0	6.6
Sulfide > 0.26 mg/L	11	**0.69**	**0.022**	**0.55**	**0.019**	0.69	0.02	-9.9	6.9
Sulfide < 0.26 mg/L	14	0.36	0.200	0.26	0.100	0.48	0.08	–	–

[1]Stations BNC-39 and -71 in August 2008 are not included in either category because sulfide concentrations from duplicate cores produced different sulfide categories.

[2]Reducing and highly reducing conditions.

[3]Moderately and weakly reducing conditions.
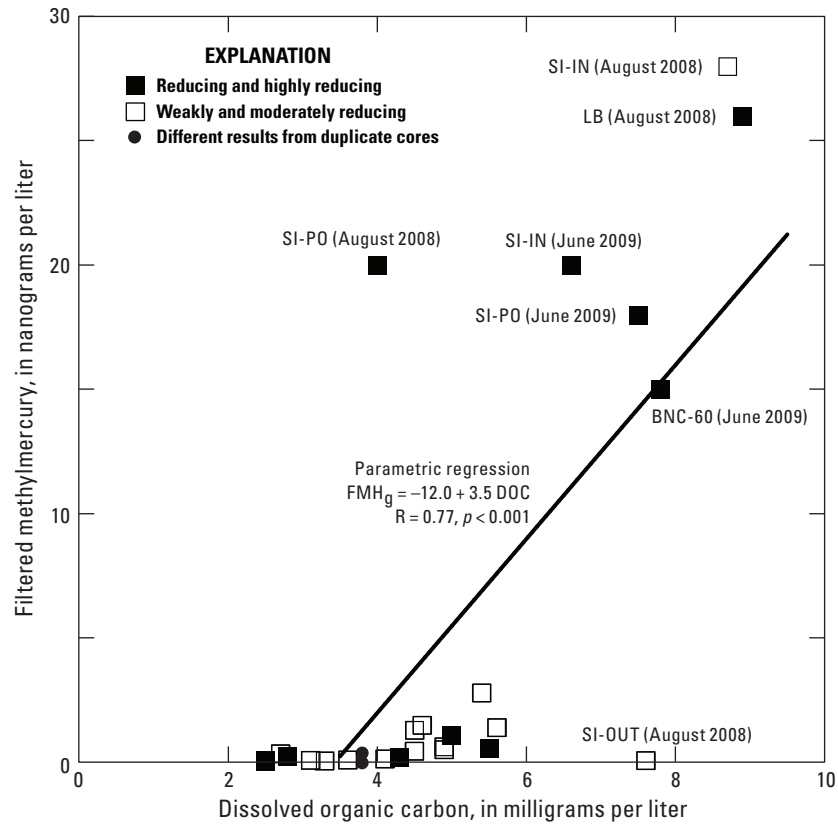
Figure 19.   Filtered methylmercury concentrations in porewaters compared to dissolved organic carbon concentrations grouped by two redox conditions, Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009. Sample with filtered methylmercury concentrations greater than 10 ng/L and samples separated from the regression line are labeled.

Table 7. Statistics for filtered total mercury, methylmercury, percentage of methylmercury, and dissolved organic carbon categorized by redox state, Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

[**Abbreviations:** mg/L, milligram per liter; ng/L, nanogram per liter; <, less than]

| Redox condition	Number of samples	Mean	Standard deviation	Median	Range	Tukey multi-comparison category
Filtered total mercury (ng/L)						
Highly reducing	2	47	15.7	47	35.9–58.1	A
Reducing	11	14.23	16.77	6.21	2.23–47.3	AB
Moderately reducing	10	15.69	16.69	10.39	3.19–59.8	B
Weakly reducing	4	6.15	3.43	5.37	2.95–10.9	B
Filtered methylmercury (ng/L)						
Highly reducing	2	23	4.24	23	20–26	A
Reducing	11	5.06	8.18	0.36	<0.04–20	B
Moderately reducing	10	3.41	8.66	0.58	0.08–28	B
Weakly reducing	4	0.83	1.32	0.23	0.07–2.8	B
Methylmercury (percent)						
Highly reducing	2	50.2	7.7	50.2	44.8–55.7	A
Reducing	11	18.8	20	8.8	0.5–62.3	A
Moderately reducing	10	11.5	13.6	8.2	0.8–46.8	A
Weakly reducing	4	18.9	28.8	6.7	0.9–61.4	A
Dissolved organic carbon (mg/L)						
Highly reducing	2	6.5	3.5	6.5	4–8.9	A
Reducing	11	4.7	1.9	4.3	2.5–7.8	A
Moderately reducing	10	5.3	1.7	4.8	3.1–8.7	A
Weakly reducing	4	3.8	1.2	3.5	2.7–5.4	A

Comparison of Porewater Concentrations with Fluxes

Sediment geochemical principles indicate that the diffusive flux of any constituent out of the sediment is proportional to its vertical concentration gradient below the sediment-water interface (Li and Gregory, 1974). The proportionality constant in an abiotic system is based on sediment texture and the molecular diffusivity of the element of interest. Organisms living in and on the sediment can inhibit or enhance the abiotic flux by a number of physical and biological mechanisms. The data from this project were examined for consistency with these principles by plotting the fluxes of FTHg and FMHg from the core incubation experiments versus the differences in concentrations between porewater and bottom waters at each station. This estimate of gradient is the only measure available from the data collected and may be imprecise estimate of the actual gradient at the sediment-water interface.

Generally, reducing and highly reducing sediments with gradients in FMHg concentration greater than 10 ng/L (SI-PO, August 2008 and June 2009; LB, August 2008; SI-IN, June 2009; BNC-60, June 2009) resulted in fluxes of FMHg greater than 200 (ng/m^2)/d (fig. 20). The anomalous low release from moderately reducing sediment from SI-IN collected in August 2008 was near the detection limit in this single core incubation experiment, even though sediment from this station had the highest porewater FMHg concentrations of the study. As indicated by the variation in the first-day release on core incubation experiment triplicates and the variation over the course of the experiment (fig. 13) among replicate cores, the variation in flux from multiple cores for a site can be high. If triplicate core incubation experiments were done in August 2008, similar high, but variable, fluxes may have been observed, which suggests that porewater FMHg may be demethylated at the sediment-water interface by benthic infauna (Choe and others, 2004).

In contrast, FMHg fluxes greater than 200 (ng/m^2)/d were measured in August 2009 in weakly reducing sediment collected from SI-PO and SI-IN in which porewater FMHg concentrations were less than 3 ng/L. Benthic infauna may enhance or inhibit the transfer of FTHg and FMHg from the sediment to the water column (Benoit and others, 2009). The lack of correlations between fluxes measured during core incubation experiments and the porewater concentrations in the top 2 cm may be a result of significant horizontal heterogeneity in sediments. Additionally, 2-cm section may not provide the vertical resolution to measure the actual concentration gradient at the sediment-water interface.
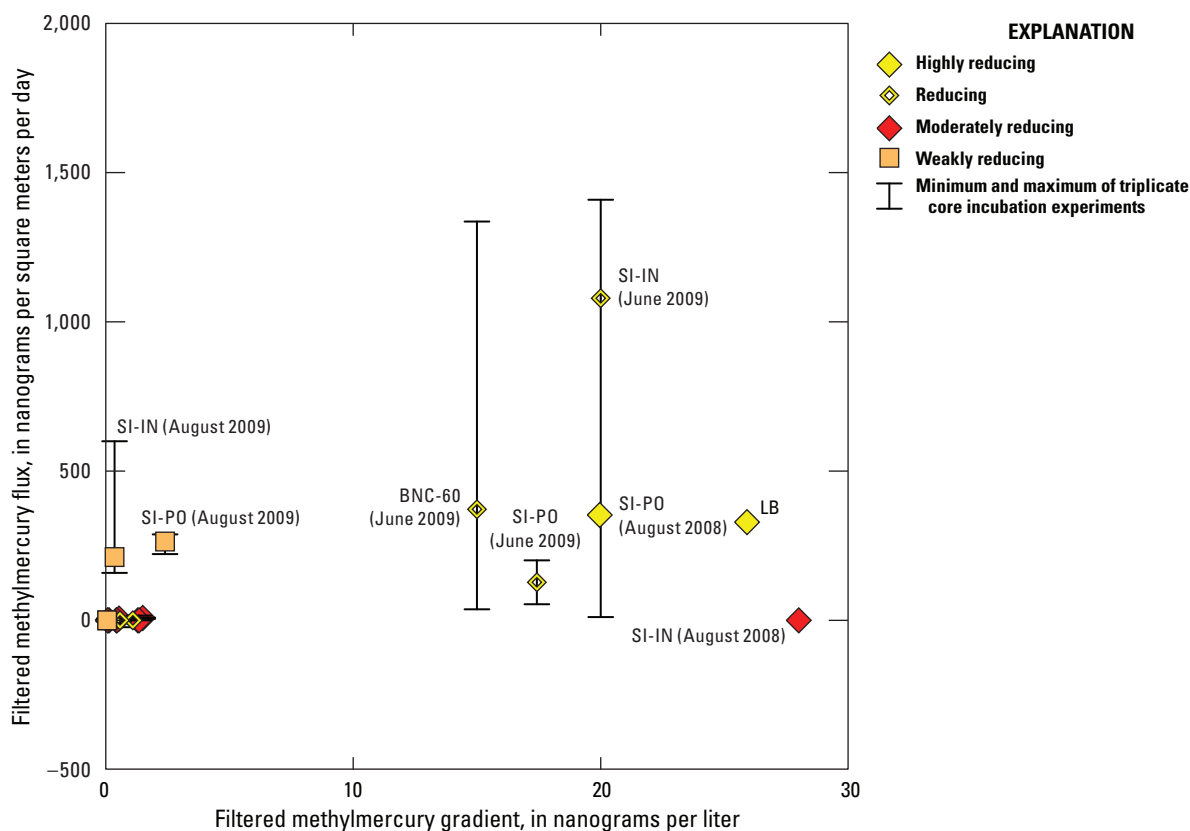
Figure 20.   Fluxes of filtered methylmercury compared to gradients of filtered methylmercury between porewater and the water column, Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

Factors Controlling Porewater Concentrations and Fluxes

The most conspicuous feature of the seasonal data is the near total absence of MHg in porewater, bottom water and surface water in Sinclair Inlet during winter (February 2009, figs. 12 and 15). In August 2008, June 2009, and August 2009, methylation in sediment of some stations led to increased concentrations of FMHg in porewaters (fig. 12). Based on these observations, analyses of variance (ANOVA) were done using models based on categorical variables of time (four seasonal events) and space (table 8). In the first model in which general area (greater Sinclair Inlet and OU B Marine) was the spatial categorical variable, the only significant relation for season was DOC. Season or area was not correlated with any mercury concentration or release value.

In the second spatial model, the six individual stations were used as values for the categorical spatial variable. In this model, season was a significant parameter for porewater DOC, FMHg concentrations and percentage of FMHg, whereas site was only a significant variable for percentage of

FMHg. Releases during the core incubation and tumbling core experiments were not correlated with either season or site.

Because DOC was always correlated with season in these temporal-spatial models, season may be a surrogate for the geochemical variable DOC. Because the correlation of porewater FTHg and FMHg with DOC differed by porewater sulfide concentrations category (table 6), a model based on the geochemical parameters DOC and the redox condition of each of the 27 sample collections (n = 27) was developed. The FTHg and FMHg in porewater were correlated with both DOC and redox; and the geochemical-based event model produced the best simulations. Although the percentage of mercury in the MHg form was correlated with DOC and redox, the geochemical based event model did not produce the best prediction. The fluxes measured during the core incubation experiments were not correlated with either DOC or redox porewater conditions, and the standard error of the predictions were only slightly lower than those of the site model. The geochemical model was not applied to the tumbling experiment because DOC and redox conditions during tumbling were considerably different from isolated cores.

Table 8.   Analysis of variance of porewater concentrations and releases of mercury from sediment in Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

[Bold values indicate statistically significant difference. **Parameter:** DOC, dissolved organic carbon; FTHg, filtered total mercury; FMHg, filtered methylmercury; %FMHg, percentage of filtered methylmercury. **f:** model a function (what is "a")?. **p:** probability of being correlation to DOC. **Abbreviations:** redox, reduction-oxidation; >, greater than; –, not measured]

Parameter	*f* (season, area)			*f* (season, site)			*f* (DOC, redox)		
	p (season)	*p* (area)	Standard error	*p* (season)	*p* (site)	Standard error	Dissolved organic carbon	Redox	Standard error
DOC	**0.01**	0.44	1.45	**0.021**	0.95	1.59	–	–	–
FTHg	0.11	0.22	14.9	0.11	0.43	15	**0.0004**	**0.034**	10.1
FMHg	0.09	0.07	7.3	**0.04**	0.069	6.5	**> 0.0001**	**0.0005**	4.2
%FMHg	0.15	0.09	18.8	**0.019**	**0.0033**	12.9	**0.0005**	**0.008**	13.5
Flux FTHg	0.23	0.18	190	0.19	0.15	230	0.3	0.07	220
Flux FMHg	0.37	0.34	240	0.38	0.32	180	0.18	0.11	160
Tum FTHg	0.6	0.76	0.48	0.74	0.64	0.50	–	–	–
Tum FMHg	0.11	0.82	0.21	0.11	0.45	0.21	–	–	–

IV.   Methylmercury Accumulation in the Base of an Estuarine Food Web

By P.W. Moran, A.R. Stewart, J. Toft, J. Cordell, and A.J. Paulson

Concern over mercury concentrations in finfish from Sinclair Inlet has prompted a more detailed investigation into the patterns of methylmercury accumulation in the base of the marine food web in and around Sinclair Inlet. As recognized by the research community, and more recently by U.S. Environmental Protection Agency (2010) with regard to new methylmercury water-quality criteria, bioaccumulation from water into increasingly higher trophic levels exerts a primary control over the concentration of methylmercury in fish and shellfish tissue (see section, "Introduction and Methods"). The largest of these bioaccumulation steps occurs in the lowest level of the food web (Pickhardt and others, 2005; Mason and others, 2006; Stewart and others, 2008). In response to Task 3 in section, "Purpose and Scope," a monthly sampling regime was implemented to evaluate the temporal and spatial variability in methylmercury accumulation in suspended solids and zooplankton of Sinclair Inlet.

The food webs of the neritic zone of Puget Sound embayments are characterized as a classic phytoplankton-to-zooplankton-to-planktivore system with strong seasonal swings in abundance, and, relative to adjacent benthic or littoral food webs, tightly coupled temporal relations (Simenstad and others, 1979; Strickland, 1983; Olson and others, 2001). Calanoid copepods have been repeatedly shown to be a dominant consumer of phytoplankton in Puget Sound (Simenstad and others, 1979; Dagg and others, 1998; Olson and others, 2001); however, they also consume ciliates in the heterotrophic cycle of bacteria or ultraphytoplankton to ciliates (Cowlishaw, 2004), and some larger types of calanoid copepods are known to be predatory on smaller copepods. The life histories of copepods common to Puget Sound also show (1) extensive use of diapause periods followed by growth and reproduction periods coincident with phytoplankton bloom seasons, and (2) more limited, low-density persistence at depth across years (Osgood and Frost, 1994). The dual characteristics of both responsive to changes in primary production and yet more persistent, and their key role as the primary food for forage and juvenile fish (Simenstad and others, 1977; Simenstad and others, 1979; Penttila, 2007), suggests that copepods make a key sentinel species for monitoring bioaccumulative compounds such as mercury. A one-time spatial sampling in Sinclair Inlet and

three representative bays (fig. 1) was completed in August 2008. From August 2008 through August 2009, four stations in Sinclair Inlet were sampled monthly (fig. 5 and, specifically, sites Sinclair Inlet, SI-IN, SI-PO, BNC-52, and the CZ).

Methods for Food Web Study

Water-Column Sample Processing and Laboratory Analysis

Dissolved organic carbon in filtrate from the TPCN methods (see section, "Release of Mercury Species from Sediment to Water Column") was analyzed at the NWQL using ultraviolet (UV)-promoted persulfate oxidation and infrared spectrometry methods (U.S. Environmental Protection Agency, 1997). The 25-mm diameter filters holding suspended solids were subsampled with a 6.3-mm diameter hole-punch (two per 25-mm filter). To prepare suspended solids samples for isotopic analysis, sub-sampled filters were moistened with Milli-Q® water (approximately 100 microliters [μL]) and fumed overnight in a desiccator with concentrated HCl to remove carbonates. The following morning, subsamples were dried in an oven at 60 °C for 1 hour and then cooled in a desiccator. The subsamples from each 25-mm filter were then loaded into tin capsules and held in desiccators until analyzed for carbon and nitrogen isotopes.

Duplicate chlorophyll samples were filtered within 2 hours of collection onto precombusted, 25-mm-diameter, glass fiber filters (nominal pore size of 0.7 μm), frozen on dry ice, and then either stored briefly at -20 °C, or placed in longer term storage at -80 °C. Chlorophyll *a* concentrations were determined fluorometrically from the 25-mm filters (Holm-Hansen and Riemann, 1978) within 6 months of collection by the USGS NRP laboratory in Menlo Park, California. The reporting level was 0.1 μg/L; values less than 1.0 μg/L were qualified as estimated.

Zooplankton Sample Collection

Live zooplankton samples returned to the laboratory and were immediately sorted into whole community and species-specific zooplankton samples for mercury analyses. Zooplankton sorting and taxonomy was done by the Wetland Ecosystem Team in the School of Aquatic and Fishery Science at the University of Washington. Species and bulk zooplankton identification and sorting was completed by trained technicians under low power magnification.

Material from the 0.1 mm mesh net was used for obtaining "whole community" samples. For these community samples, zooplankton were first separated from phytoplankton

as much as possible by swirling aliquots of the sample in a petri dish, causing heavier diatoms to separate from the lighter zooplankton. Representative samples of the zooplankton then were moved using plastic forceps onto 0.073-mm mesh pieces of net and concentrated for freezing and later analysis. When approximately 10–12 milligrams (mg) wet weight of organisms were obtained, they were placed on a small sheet of filter paper (about 5 × 8 mm), put into labeled 1.5-mL centrifuge tubes, and frozen for later analysis.

The 0.253-mm mesh samples were used to obtain individuals of larger species for analysis. The most abundant organism was used (for example, calanoid copepods [*Acartia* sp.]). If possible, the same species was used across all sampling stations for a given date, but this was not often possible because of low numbers of the selected organism at one or two stations. Individual species were collected by pipet from the sample jar, placed on 0.073-mm mesh pieces of net, and individually sorted until approximately 10–12 mg wet weight of a given species was obtained; species were then frozen using the same method as for the whole community samples. Vertical hauls that had been fixed in the field were quantitatively subsampled if necessary in the laboratory, using a Hensen's Stempel pipette to obtain approximately 200 of the most abundant taxon. Plankton taxa were enumerated and all adult copepods were identified to genus or species.

Composite samples of zooplankton representing either the whole community or individual species were freeze-dried (using a Virtis Genesis 35EL) prior to processing for stable isotope and MHg analysis. Zooplankton samples for stable isotopes were weighed using a microbalance (1–2 mg) and packed into tin capsules.

Stable isotopic analysis of the suspended solids and zooplankton was completed on frozen samples by the University of California at Davis Stable Isotope Facility (University of California, 2011) following established methods. Suspended solids samples were evaluated in triplicate; zooplankton replication depended on organism availability, but ranged from two to five replicates per sampling station and time. Isotope ratios of carbon ($\delta^{13}C$) and nitrogen (and $\delta^{15}N$) of the suspended solids and zooplankton were determined using a Europa Scientific Hydra 20/20 continuous flow isotope ratio mass spectrometer in conjunction with a Europa ANCA-SL elemental analyzer to convert organic carbon and nitrogen into carbon dioxide and N_2 gas. Nitrogen isotope samples were standardized against N_2 in air as follows:

$$\delta^{15}N\ ‰ = [(Rsample/Rstandard) - 1] \times 1{,}000, \quad (6)$$

where

R (ratio)equals $^{15}N/^{14}N$, and

‰   is parts per thousand.

Likewise, $\delta^{13}C$ (R = $^{13}C/^{12}C$) or (carbon with atomic weight of 13/carbon with an atomic weight of 12) with a similar formula as above is reported here as a standardized carbon isotope ratio against the international standard, Vienna Pee Dee Belemnite.

Methylmercury concentrations in whole community and species-specific zooplankton samples were determined by the USGS Mercury Laboratory in Menlo Park, California. Subsamples of freeze-dried zooplankton (whole community and species specific) were weighed by using a microbalance (1–2 mg) into 1.5 mL acid-washed centrifuge tubes. Methylmercury was extracted from the samples by adding 200 µL of 25-percent (weight/volume) KOH/methanol to the centrifuge tubes. The sample and extractant were homogenized by vortexing and then samples were heated in an oven at 60 °C for 4 hours, with samples being vortexed once after 2 hours. Digested samples were cooled and stored frozen (-80 °C) until analysis. On the day of analysis, samples were thawed, and 8 µL of low-DOC laboratory water was added to each centrifuge tube. The samples were then homogenized by vortexing and then centrifuged at 4,000 rpm for 15 minutes to compress the remaining solid material into a pellet. A 0.15-mL aliquot of the extractant was subsampled into a trace-metal-clean glass I-Chem™ vial along with a drop (about 25 µL) of a silica-based antifoaming agent. The vial was nearly filled with laboratory water, the pH was adjusted to 4.9 by using acetate buffer, and an ethylated agent (sodium tetraethyl borate) was added. The vial then was topped off with laboratory water, capped with a septa screw-top cap, and shaken. MHg was converted in the vial to volatile methyl-ethyl-mercury, which was subsequently analyzed by MERX Automated Methyl Mercury Analytical System (Brooks Rand Laboratories, Seattle, Washington) by using CVAFS detection. Each batch of analytical samples was accompanied with analysis of the minimum following quality assurance samples: (1) two certified reference materials—National Research Council Canada DORM-2 Dogfish muscle and NIST-Research Material 2890 Mussel Tissue, (2) one matrix spike sample, (3) one analytical duplicate, and (4) one method blank. Mercury analysis in water and filtered particulates (solids) is discussed in section "Release of Mercury Species from Sediment to Water Column."

Results of Food Web Study

Spatial Sampling in August 2008

With the exception of chlorophyll, the synoptic sampling during August 2008 showed water column constituents associated with carbon to be relatively consistent at all stations (fig. 21). Chlorophyll, in contrast, was variable across stations, and had significantly higher concentrations (one-way
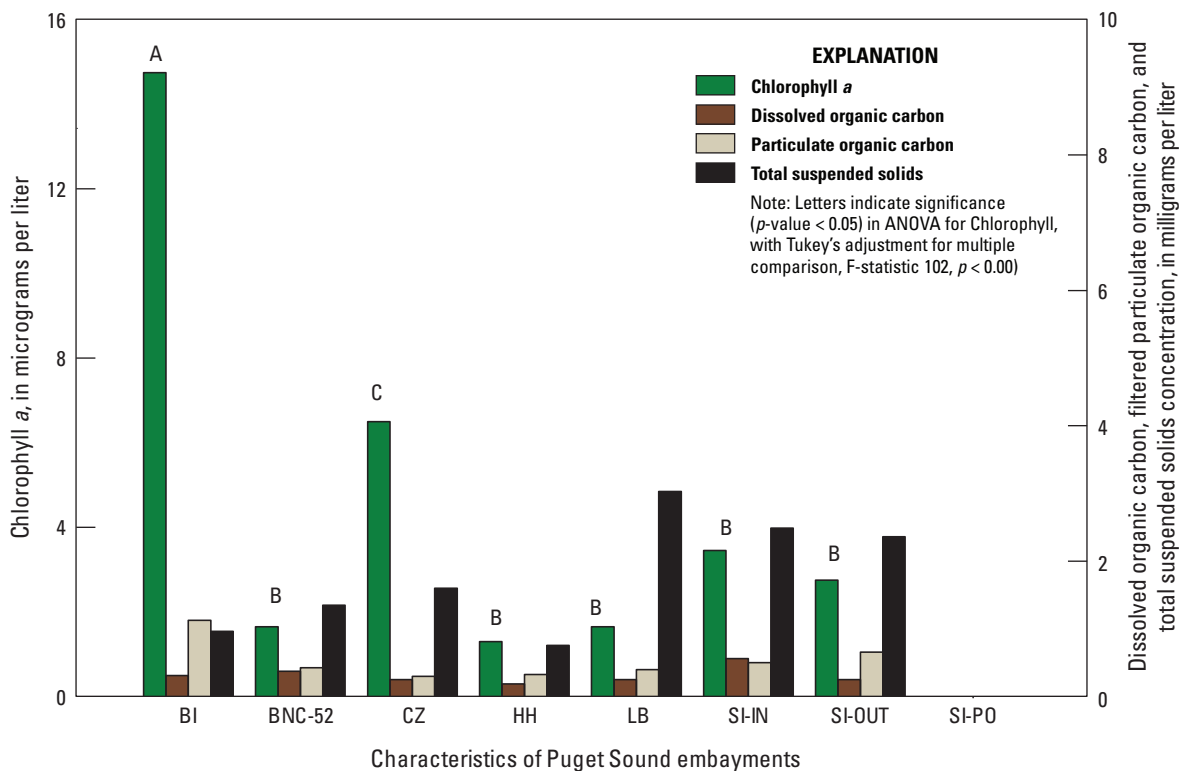
Figure 21.   Concentrations of selected surface-water constituents associated with carbon, Puget Sound, Washington, August 2008. Stations include three representative bays (Budd Inlet [BI]; Holmes Harbor [HH]; and Liberty Bay [LB]); and five greater Sinclair Inlet (SI) stations: (OU B- Marine station (Bremerton naval complex [BNC-52]), CZ (convergence zone), Inner [SI-IN], Outer [SI-OUT], and Port Orchard [SI-PO]. No samples were collected at SI-PO in August 2008.

ANOVA with Tukey's adjustment for multiple comparisons, F-statistic of 102, p-value <0.01) at BI, followed by the CZ, as compared to other study sites in the large-scale spatial comparison of August 2008. Intense spring and summer algal blooms observed during this study period also were described in Winters and others (1975), with phytoplankton production rates of 460–470 grams of carbon per square centimeter per year reported in central Puget Sound. The largest chlorophyll values in August 2008 were from BI and were consistent with the warmest temperature (22.3 °C) and highest orthophosphate concentration (0.135 mg/L) (see Huffman and others, 2012, appendix G), relative to all stations sampled in August 2008

(n=7). Similarly, total particulate carbon (table 1-4) and particulate organic carbon concentrations (fig. 21) were highest in Budd Inlet during that month. However, nitrate, as nitrate plus nitrite, did not differ substantially between the seven sites in August 2008 (see Huffman and others, 2012, appendix G). Dissolved organic carbon was unremarkable at Budd Inlet and fairly consistent across all stations in August 2008, ranging from less than 0.4 to 0.9 mg/L. Zooplankton tissue concentrations of MHg were not significantly different across the stations in August 2008 (Scheffe post hoc test, ANOVA, F-ratio 24.2, p-value < 0.01; fig. 22).
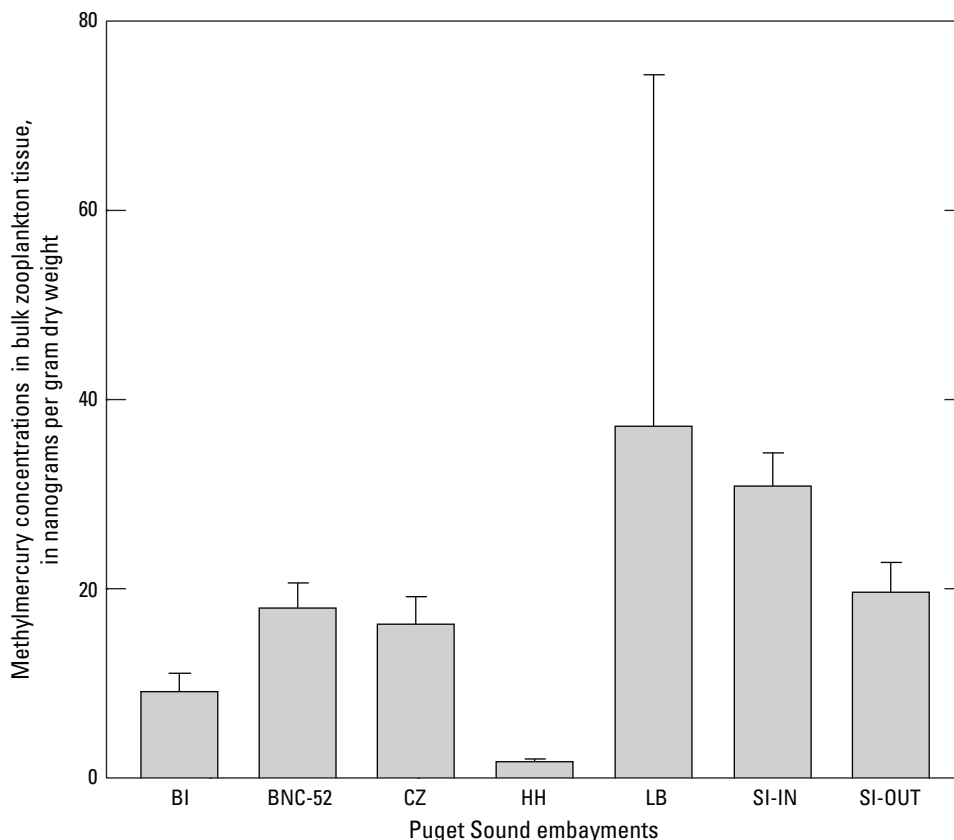
Figure 22.   Concentrations of methylmercury in bulk zooplankton tissue, Sinclair Inlet, Kitsap County, Washington, August 2008. Stations include three representative bays (Budd Inlet [BI], Holmes Harbor [HH], and Liberty Bay [LB]); greater Sinclair Inlet (SI) stations (OU B- Marine station (Bremerton naval complex [BNC-52], CZ, convergence zone Inner [SI-IN], and Outer [SI-OUT]).

Despite a lack of significant difference in MHg concentrations in zooplankton tissue collected from the seven stations in August 2008, stable isotope signatures of $\delta^{15}N$ and $\delta^{13}C$ indicate significant spatial differences in carbon and nitrogen sources (fig. 23). In particular, the greatest relative degree of separation is for Budd Inlet and Holmes Harbor, which are spatially the most distant stations. Intermediate between them, primarily in ^{13}C values, are the SI stations and the adjacent LB station. Although the concentrations from the CZ and LB stations plot near the SI stations, they are somewhat distinctive in this group of five. This grouping is consistent with spatial differences and the understanding

of tidal exchange dynamics in the area. That is, the location of the CZ just outside the mouth of Sinclair Inlet, at the intersection of Port Washington Narrows and Rich Passage (figs. 1 and 5), has considerably greater tidal exchange than the three more western SI stations (Gartner and others, 1998; Wang and Richter, 1999).

As demonstrated in the studies of Kidd and others (1995) and Stewart and others (2008), there often is a relation between the ^{15}N value of an organism and its methylmercury concentration. This relation is somewhat evident, although not statistically significant, in samples collected in August 2008 (fig. 23); (spearman rank correlation of -0.61, p-value =0.34).
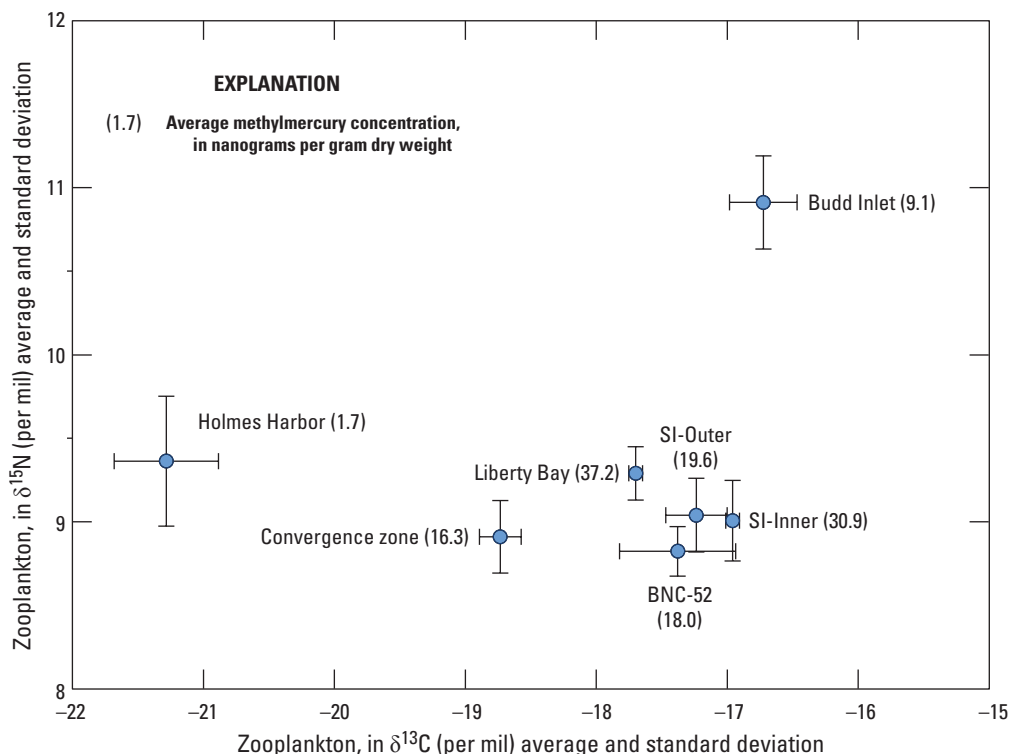
Figure 23.   Comparison of stable nitrogen isotope (δ^{15}N) and stable carbon isotope of (δ^{13}C) ratios in zooplankton tissue for stations in Sinclair Inlet and representative bays, Puget Sound, Washington, August 2008.

Monthly Sampling in Sinclair Inlet

Approximately monthly (December 2008 was not sampled) samples were collected between August 2008 and August 2009 at the four Sinclair Inlet stations: SI-IN, SI-PO, BNC-52, and the CZ (figs. 5). This sampling included numerous water column constituents, four mercury constituents in water and the collection, identification, sorting, and sub-sampling of zooplankton for tissue concentrations. These monthly samples also corresponded to the quarterly benthic sediment sampling. Strong seasonal trends in chlorophyll were measured and visually observed in summer, at the four Sinclair Inlet stations, with concentrations ranging from less than 1 µg/L in middle of winter to 150 µg/L in late summer (fig. 24).

Strong and spatially similar phytoplankton growth in Sinclair Inlet during spring and late summer is indicated in figure 24 (see "Seasonal Analysis"). This growth phase is then interrupted with abrupt "crashes" in chlorophyll concentrations in mid- to late summer followed by rebounds in concentrations. This classic phytoplankton bloom and chlorophyll crash pattern previously has been described in Puget Sound (Winters and others, 1975; Strickland, 1983). Although adequate data were not available preceding the August 2008 sampling to confirm the phenomena, the autumn "bloom" or apparent rebound in the September 2008 chlorophyll samples (fig. 24) is reflected in the methylmercury concentrations of suspended solids from August to October 2008 (fig. 25).
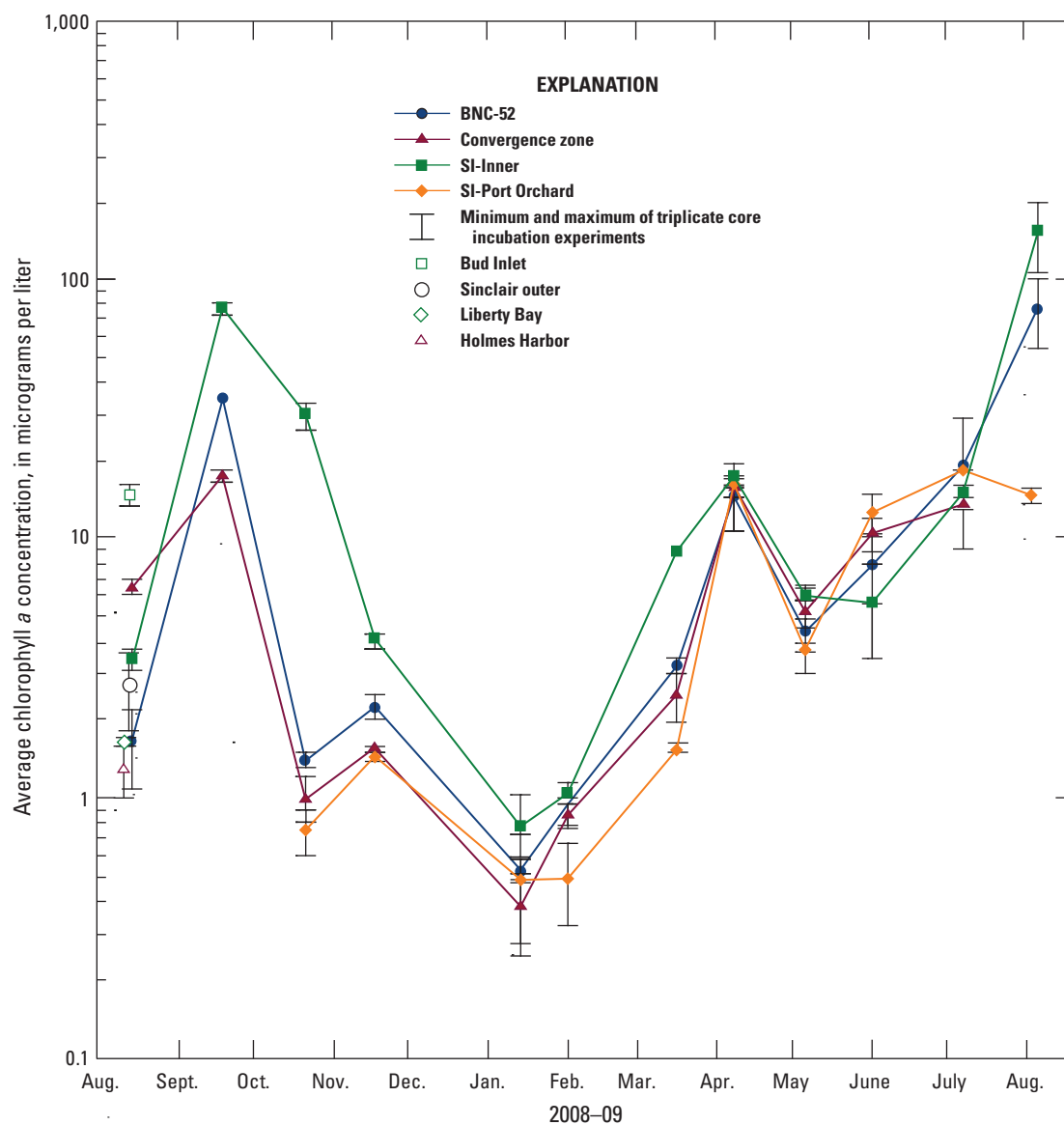
Figure 24.   Average chlorophyll *a* concentrations for selected stations in and adjacent to, Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.
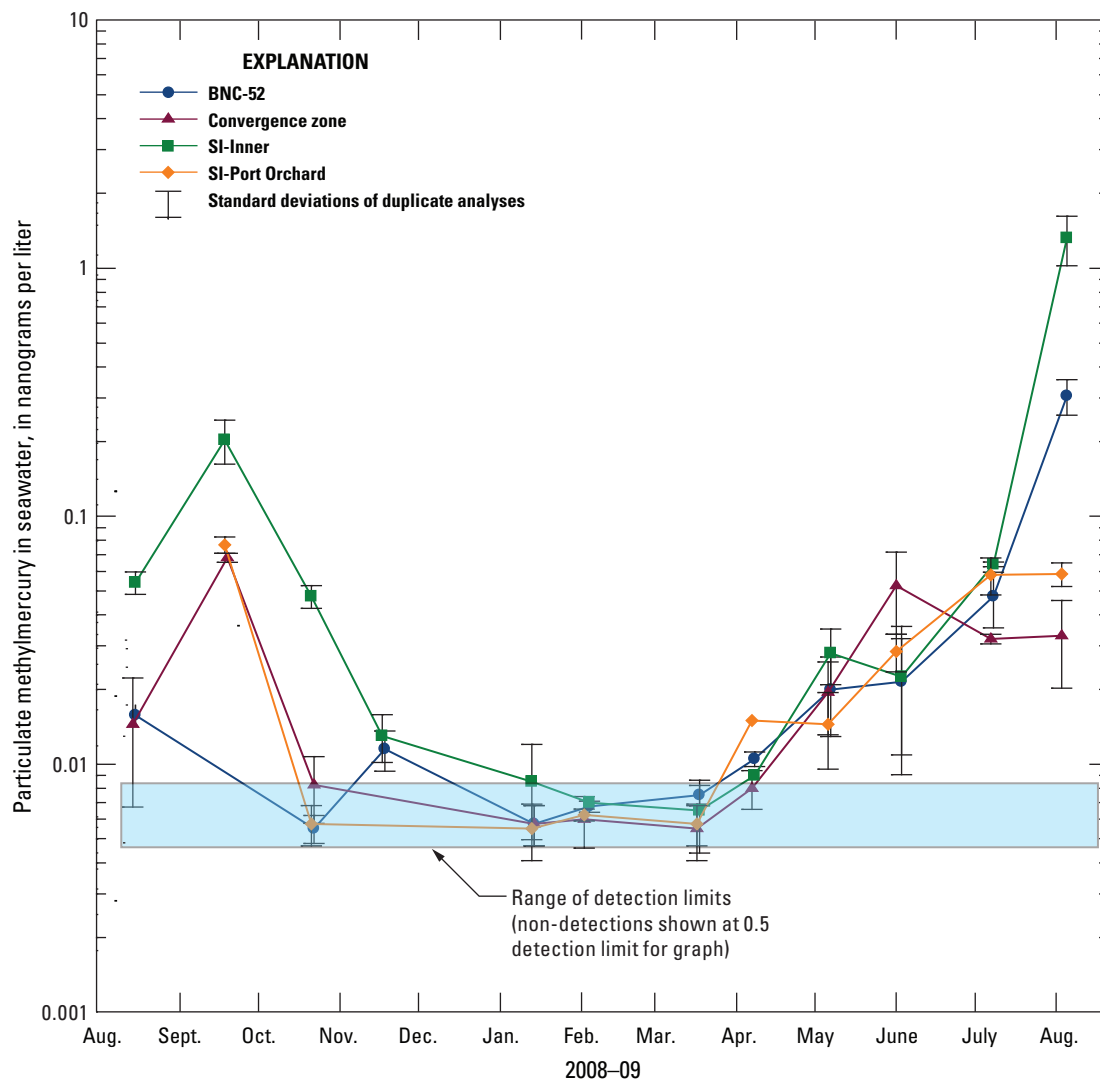
Figure 25.   Particulate methylmercury (mass/volume) concentrations for selected stations in and adjacent to Sinclair Inlet, Kitsap County, Washington. Data are logarithmic transformed.

The coupling of the overall pattern of increasing mercury concentrations as phytoplankton concentrations increase has been described in several freshwater systems (Miles and others, 2001; Stewart and others, 2008), but the phenomena in coastal marine waters is less well described. This seasonal trend in increases of methylmercury during the growing season is also visible, although less clear, when evaluating the concentration of filtered methylmercury in seawater over the sampling period (fig. 26).

Similar to the strong seasonal trends in chlorophyll (fig. 24) and dissolved nutrients (fig. 1-2), the ratio of ^{15}N to ^{14}N of suspended solids increased during the growing season, suggesting an increased coupling of the nutrient cycle in the water column. These seasonal trends are seen at four Sinclair Inlet stations. An anomaly to this trend is SI-IN in February 2009 with a significant decrease in the ^{15}N ratio (fig. 27).

In February 2009, SI-IN (fig. 1-2A) also had dramatically higher filtered ammonia values (0.22 mg/L compared to an average of 0.03 mg/L for the other 11 months) and a filtered orthophosphate value of 0.11 mg/L (fig. 1-2B) that was the highest measured for any of the stations during that month, and the third highest in this study. Orthophosphate values at the other three stations ranged from 0.08 to 0.09 mg/L in February 2009. These observations suggest an additional nutrient source was present during the February 2009 sampling. A review of the tidal and weather data for February 2009 indicates nothing remarkable.
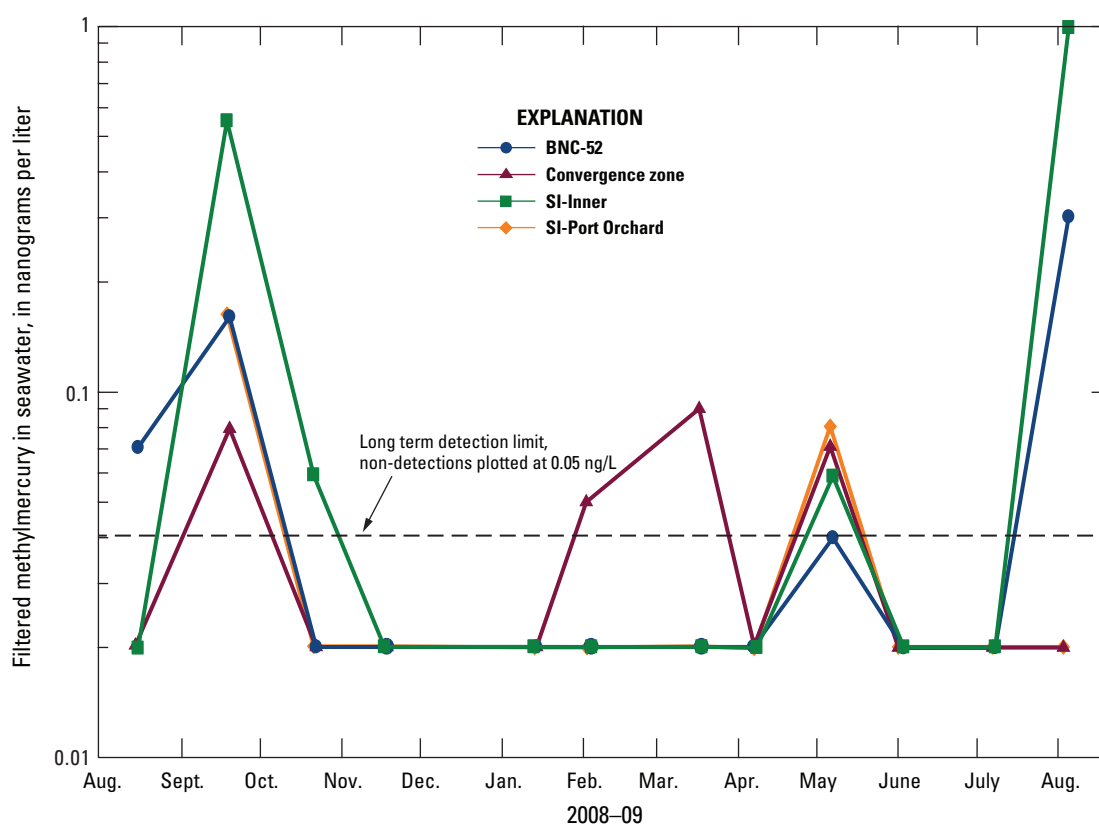
Figure 26.   Filtered methylmercury concentrations in seawater for selected stations in and adjacent to Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.
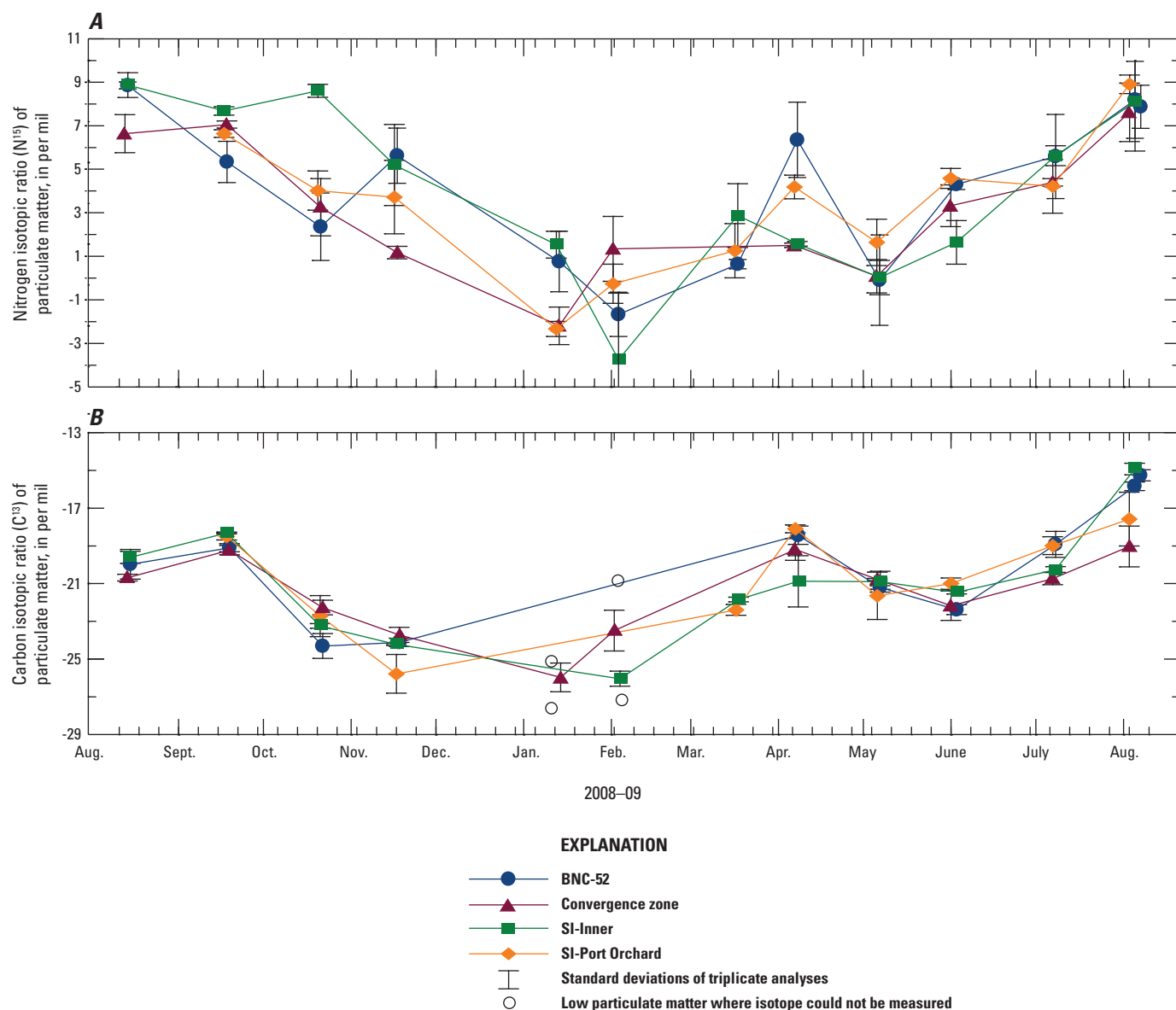
**Figure 27.** Average ratios of stable isotopes of (*A*) nitrogen (δ^{15}N) and (*B*) carbon (δ^{13}C) in suspended solids for selected stations in and adjacent to Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

Estimating Zooplankton Mercury

In addition to the monthly sampling of nutrients and chlorophyll, other parameters were co-sampled at the time of zooplankton collection that were expected to aid in estimating zooplankton mercury concentrations. The distribution of these explanatory parameters were reviewed and transformed as needed to meet assumptions of normality. In order to explain zooplankton tissue concentrations of MHg, several predictor variables were evaluated. Their significance in ordinary least squares regression were as follows- natural log (ln) of average chlorophyll a (multiple R^2 0.125, p-value 0.012), ln FMHg in nanograms per liter (R^2 0.30, p-value 0.019), ln of average PMHg in nanograms per liter (R^2 0.345, p-value 0.001), and average $\delta^{15}N$ (R^2 0.159, p-value 0.004), respectively. Stepwise regression also was done with a combination of two or three of these parameters, excluding FMHg or PMHg, which were correlated to zooplankton MHg (Spearman's rho of 0.54–0.52). No stepwise combination improved upon the single, average PMHg model in estimating zooplankton mercury concentrations. The importance of suspended solids to estimate the accumulation to the next trophic level has been demonstrated by Stewart and others (2008). Despite the recent examples of "bloom dilution" of methylmercury in freshwater primary consumers (Pickhardt and others, 2002; Karimi and

others, 2007), bulk zooplankton tissue concentrations of methylmercury in this study (fig. 28) generally track, albeit with a 30–45 day delay, concentrations observed in the particulate phase (fig. 25) and chlorophyll (fig. 24).

A review of the August 2008 synoptic sampling and the year-long monthly sampling results suggests that although there are some spatial differences in mercury concentrations across Puget Sound, the differences appear to be smaller, or similar in magnitude, than differences at a given site over the course of the year. The strong seasonal phytoplankton growth, as indicated by chlorophyll concentration, provides an overriding control on mercury uptake and accumulation into the lower estuarine trophic levels. These seasonal peaks in MHg concentrations are weak, but evident, in the FMHg concentrations, and stronger in the PMHg and zooplankton concentrations (fig. 29). These trends correspond to the quarterly sediment sample results that indicated production and release of methylmercury from the sediments and that increased in strength from February to June and then leveled off or decreased slightly by August. Temperature alone has strong controls on MHg production (Fagerstrom and Jernelov, 1972; Wright and Hamilton, 1982), and likely is somewhat responsible for the increase in MHg availability in sediments. Near-bottom temperatures varied from 7 to about 14 °C during the year; however, the pelagic production, senescence, and
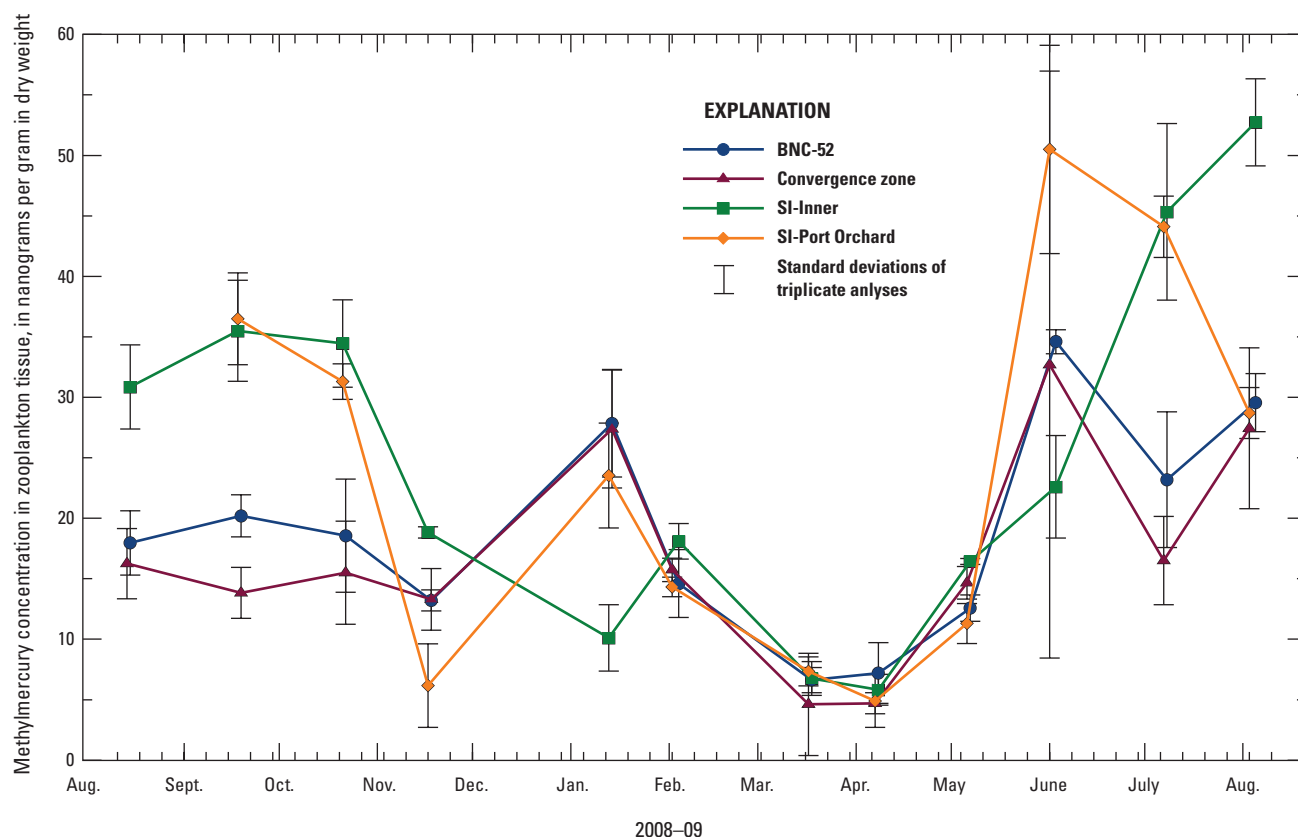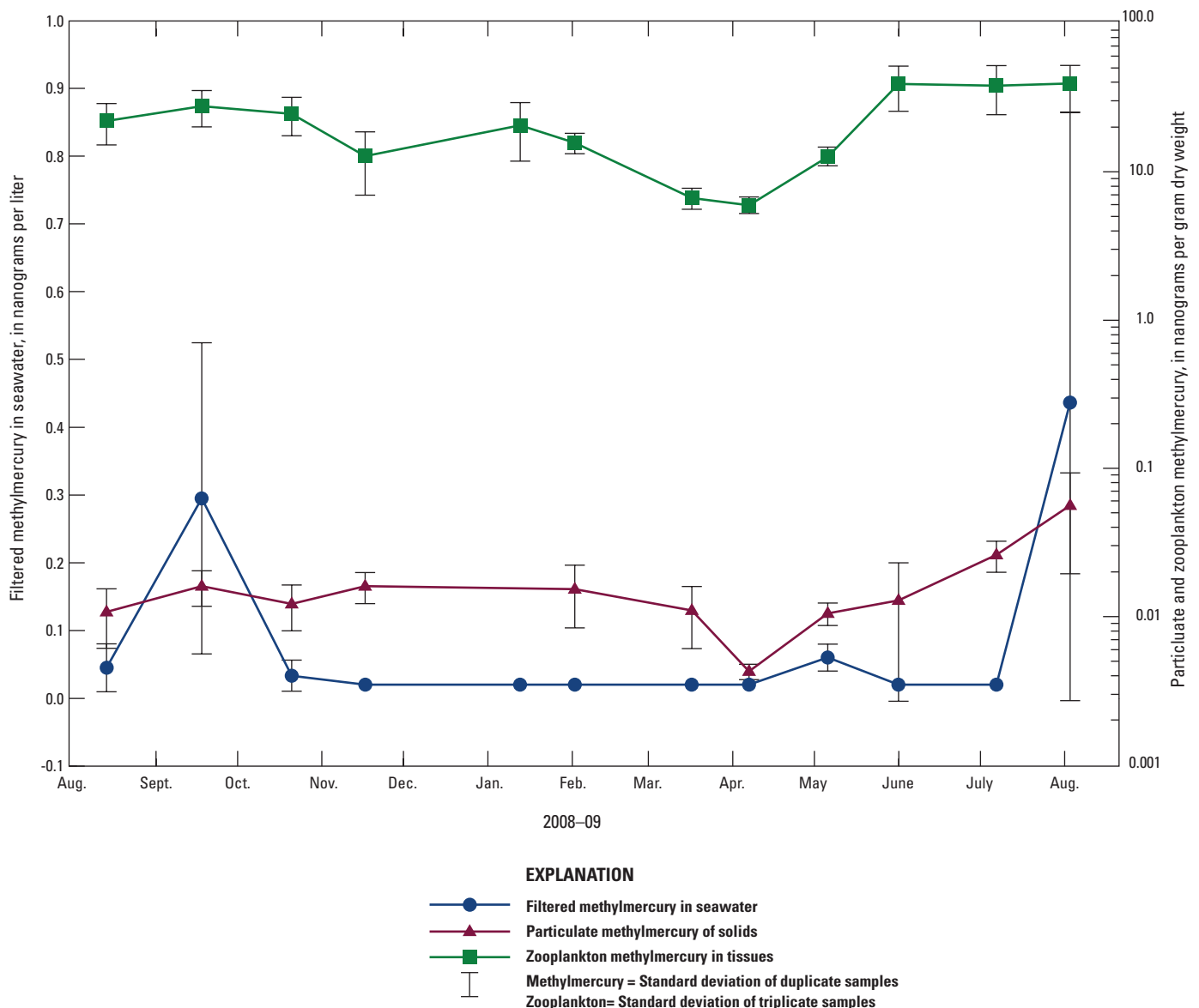

Figure 28. Average methylmercury concentrations in zooplankton tissue for selected stations in and adjacent to Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

deposition of phytoplankton to the rather shallow sediments appears to have a stimulatory effect on sulfate-reducing bacteria and, therefore, methylmercury production.

A comparison of methylmercury concentrations in particles and in zooplankton tissue for the same sampling date (fig. 29) indicates a several order of magnitude increase in concentration on a dry weight basis. This phenomenon of bioaccumulation in the marine food web has been well described; however, given the high frequency of non-detectable concentrations in the filtered water sample collected, a bioaccumulation factor from water to zooplankton could not be calculated.

The FMHg concentrations greater than 0.25 ng/L were measured in one-half of the surface water samples collected in the greater Sinclair Inlet in August 2009. The highest concentration of FMHg during this study (0.99 ng/L) was measured at SI-IN. The ancillary data indicate that the extremely high concentrations of FTHg and FMHg in surface water measured at station SI-IN in August 2009 (figs. 14–15) was associated with biological processes and high chlorophyll concentration. Thus, the phytoplankton bloom conditions in August 2009 accumulated carbon, nitrogen and phosphorus, THg, and MHg in the particulate phase. As the phytoplankton died, there is an apparent release of these elements into aqueous or colloidal phases. Nitrate is limiting and is reused to produce new phytoplankton, which leaves an accumulation of filterable orthophosphate. Colloidal THg and MHg from the phytoplankton breakdown that pass through the pores of the QFF filters used likely are responsible for the increased FTHg and FMHg concentrations during bloom conditions at SI-IN during August 2009.

Figure 29.   Averaged concentrations of filtered methylmercury in seawater, particulate material, and zooplankton for selected stations in and adjacent to Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009.

V.   Synthesis

By A.J. Paulson, P.W. Moran, and
M.C. Marvin-DiPasquale

Water column processes acting on the aqueous and particulate phases of mercury control the bioaccumulation of methylmercury into the base of the pelagic food web in Puget Sound. The concurrent annual cycling of biological indicators (chlorophyll *a*, orthophosphate, nitrate, and silicate) at each of three stations in Sinclair Inlet suggests that the near-surface water of Sinclair Inlet is well mixed by tidal dispersion. Thus, seasonal biological productivity, rather than geographical location, generally controls mercury bioaccumulation in the surface layer of Sinclair Inlet. The CZ station outside of Sinclair Inlet seems to be responding in a slightly different manner than Sinclair Inlet stations (fig. 5).

Correlations between Methylation, Release, and Bioaccumulation

The MHg concentrations of suspended solid in the near-surface water of Sinclair Inlet increased between August and September 2008 (fig. 25, and summarized in fig. 29). Likewise, the average MHg concentrations of zooplankton increased slightly (from 21 to 26.5 ng/g). Throughout winter (November 2008–March 2009), MHg concentrations of solids were low and near the detection limits. The MHg concentrations of zooplankton also decreased during winter, reaching their lowest median concentrations in April 2009 (fig. 28). Beginning in April 2009, MHg concentrations of solids increased through August 2009, the end of the sampling effort. Beginning in April, zooplankton MHg also increased and reached a plateau of about 35 ng/g zooplankton tissue in July 2009. Measurable concentrations of FMHg throughout Sinclair Inlet were detected only in October 2008 and May 2009, near or after the time of the chlorophyll maximum. Thus, MHg concentrations of suspended solids are better indicators of bioaccumulation into the food web than MHg concentrations in water, as FMHg is quickly taken adsorbed by particles. Methylmercury has particularly strong affinity for sulfhydryl groups common to biological molecules (Ravichandran, 2004). The use of MHg of suspended solids as an indicator of bioaccumulation into the food web could be further expanded by lowering the reporting limit of MHg of suspended solids simply by filtering more water.

The seasonal trends in fluxes of FMHg in sediment suggest that sedimentary processes are producing the MHg that is being accumulated in suspended solids and zooplankton. High, but also variable, fluxes of FMHg from sediments (fig. 30) were detected when MHg of suspended solids were high. The fluxes of FMHg (fig. 30) were controlled by accumulation of FMHg in porewater (fig. 31). In reducing and highly reducing sediment, porewater FMHg concentrations were highly correlated with SMHg concentrations (fig. 32). Likewise, the seasonal trends in SMHg (fig. 31) were similar to the seasonal trends in methylmercury production potential (fig. 30).

Sedimentary diagenetic processes likely are controlled by biological processes in the water column. Analysis of covariance suggests that MMP, SMHg, FMHg in porewater, and fluxes out of the sediment are primarily controlled by seasonal factors, redox-sensitive constituents, and organic carbon (figs. 10–11; tables 6–8). In turn, sedimentary redox conditions are controlled by the flux of labile carbon to the sediment, especially after a phytoplankton die-off. Baker and others (1985) demonstrated that the flux of labile carbon (concentration of pigments in sediment trap material) to the sediment-water interface of Puget Sound sediment was controlled by bloom-induced increases in pigment concentrations in the euphotic zone during short periods in June, August, and September. The increased incorporation of reduced Mn into shell of bivalves in the Bay of Seine, France, followed maximums in the water column chlorophyll *a* (Barats and others, 2008). The time history of Mn in bivalve shells, which was co-incident with high ammonia concentrations in the sediments, following phytoplankton blooms clearly demonstrates the linkage between biological process in the water column and redox conditions in the sediment.

Specifically, the high porewater FMHg concentrations and FMHg fluxes from the sediment samples collected in June 2009 may have been a result of the supply of labile carbon from the phytoplankton die-off as a remarkable decrease was observed in chlorophyll *a* concentrations in May 2009 (fig. 24). The data from four seasonal sampling periods in 2008 and 2009 are limited and somewhat variable. However, the opinion that plankton die-off stimulates microbial activity in shallow estuary systems has been described in Chesapeake Bay, Maryland (Kemp and Boynton, 1981). Therefore, the production and cessation of phytoplankton may stimulate the subsequent production of MHg in the sediment and thus its transfer back to the pelagic food web.
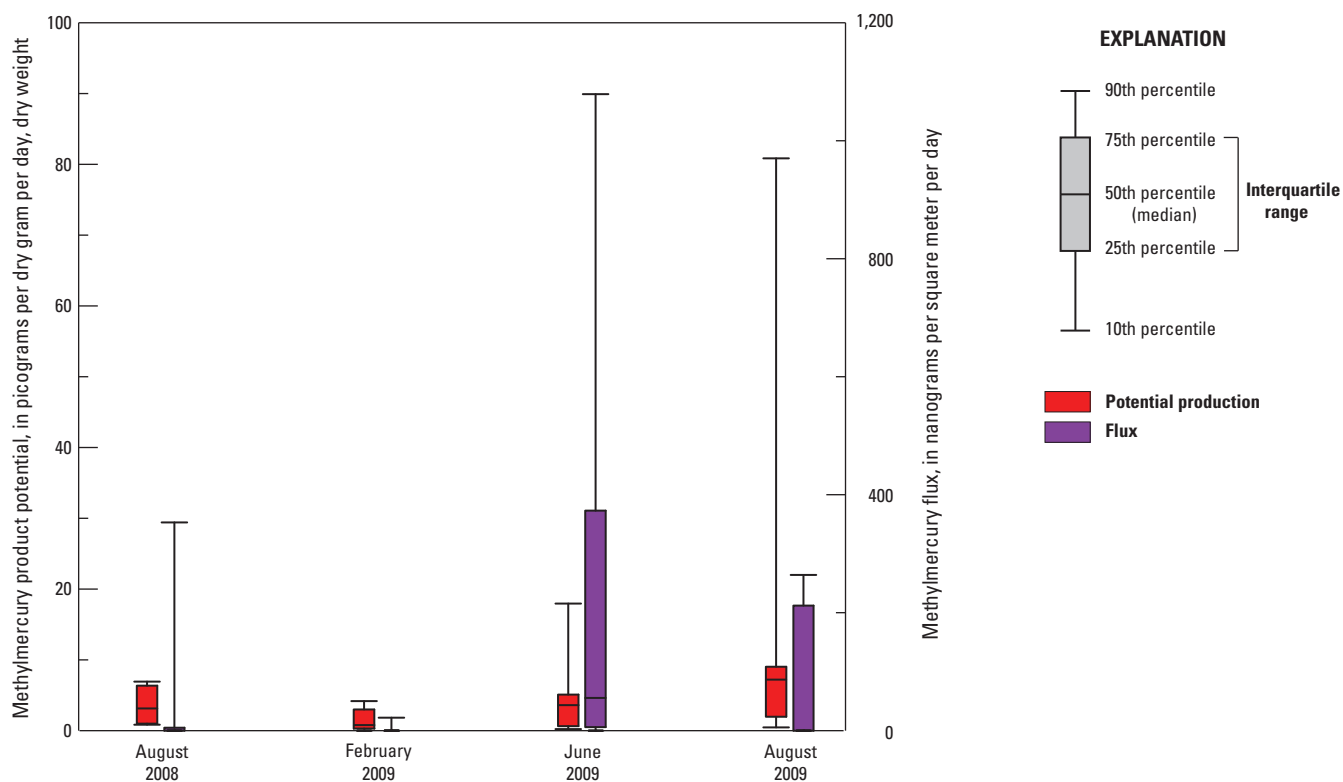
Figure 30.   Methylmercury production potential and median fluxes of sediment at OU B Marine, and greater Sinclair Inlet stations, Kitsap County, Puget Sound, Washington during seasonal sampling events, August 2008–August 2009.
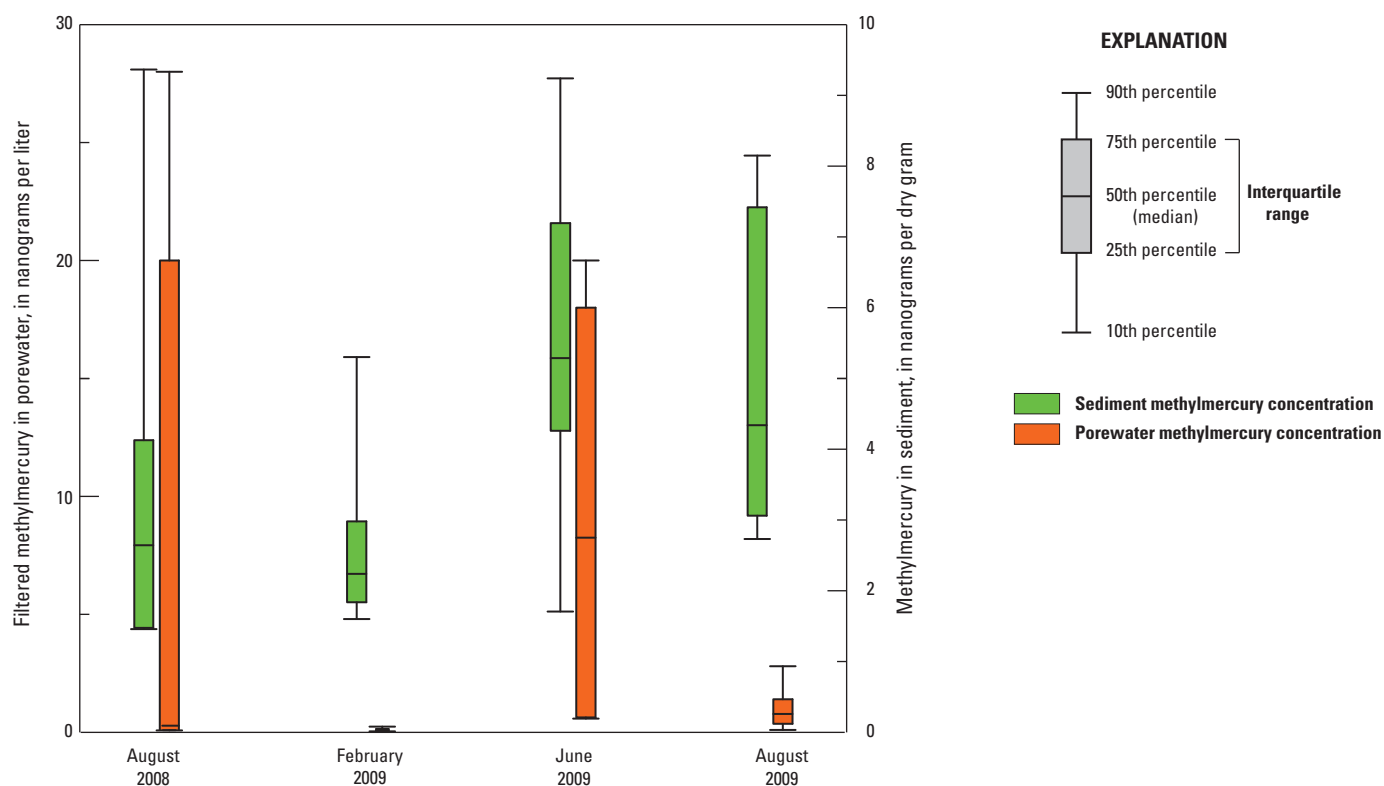
Figure 31.   Filtered methylmercury concentration in porewater and in sediment at OU B Marine, and greater Sinclair Inlet stations Kitsap County, Washington, August 2008–August 2009.
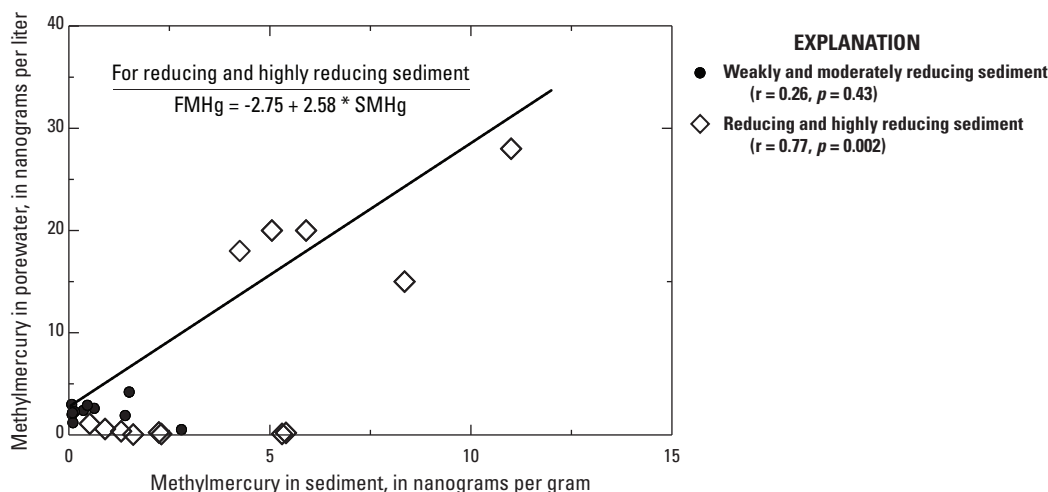
Figure 32.   Methylmercury concentrations in porewater compared to methylmercury concentrations in sediment, Sinclair Inlet, Kitsap County, Washington, August 2008–August 2009. The correlation coefficients (r) and the probabilities of the slope being about zero (*p*) for two groupings of redox conditions are given. The regression line for the more reducing grouping is shown with the significant regression.

Summary

In August 2008, samples of sediment, water, and biota from three bays representative of Puget Sound, Washington, embayments and from six stations in Sinclair Inlet were collected. The representative bays ranged from locations in Holmes Harbor on remote Whidbey Island to Liberty Bay, which was adjacent to a suburban town and contained highly reducing sediments. Substantially higher concentrations of total mercury and reactive mercury in sediment were measured in Sinclair Inlet relative to the representative bays. In contrast, there was no difference in sediment methylmercury concentrations, in methylmercury concentrations in porewater, or in the water-column methylmercury concentrations of Sinclair Inlet relative to the representative bays in August 2009. Although the inorganic reactive mercury (SRHg) metric provides some measure of the pool of inorganic mercury (Hg(II)) that is potentially available for Hg(II)-methylation, there is no evidence that methylmercury (MHg) production rates are significantly higher in Sinclair Inlet than in the representative Puget Sound embayments in this study.

Likewise, broad-scale sampling of sediments over 1 year detected significantly higher concentrations of total and reactive mercury in Bremerton naval complex (BNC) sediment compared to greater Sinclair Inlet sediment. Previous analysis of the total mercury (THg) concentrations of solids also indicated that suspended matter collected from near-bottom waters of BNC stations were higher than those of greater Sinclair Inlet (GSI). Yet, there was no difference in sediment methylmercury (SMHg) concentrations or methylmercury production potential (MPP) rates between BNC and GSI. A model that examined differences among the individual stations, rather than differences between GSI and BNC, better described the factors controlling porewater filtered methylmercury (FMHg) concentrations and methylmercury (MHg) release from sediments.

Rather than detecting differences based on geographical distinctions, these observations are consistent with the concept that sediment total mercury (STHg) has only a minor effect on net MPP rates and SMHg concentrations. The Akaike Information Criterion competitive model approach used to assess key MHg-metrics in sediments from this study support this concept. The proxy measure for the activity of the Hg(II)-methylating microbial community (methylmercury production rate constant [k_{meth}]), was best described by a function that included temperature, sediment redox, and the percentage of acid-extractable ferrous iron/total iron (sediment) $(Fe(II)_{AE}/Fe_T)$ as significant variables, consistent with the role of iron-reducing bacteria in the Hg(II)-methylation process. However, the importance of sulfate-reducing bacteria cannot be ruled out in this analysis. The proxy measure for the availability of Hg(II) to those Hg(II)-methylating bacteria across all sites, namely SRHg, was best described by a function that included sediment redox (E_h), STHg, and sediment bulk density, the latter parameter reflecting the combined influence of sediment organic content and

grain size. When representative bays were excluded from the analysis, STHg was no longer a significant variable for describing sediment SRHg concentrations. The analysis of both k_{meth} and SRHg data point to the dominant role sediment redox has on the SMHg production process. The MPP rates were a function of temperature, STHg concentration, and the percentage of $Fe(II)_{AE}/Fe_T$ across all sites, but at the local scale (GSI and BNC stations only, excluding representative bays) the importance of STHg was again eliminated from the best fitting model. Although only 35–36 percent of the variability in sediment MPP rates could be explained by the final, best fit models, 83 percent of the variability in SMHg concentration for Sinclair Inlet (GSI and BNC stations combined) was explained by sediment redox and bulk density alone.

Methylmercury concentrations in sediment porewaters also were correlated with dissolved organic carbon, and the correlation was especially strong for reducing and highly reducing sediments. The correlation between MHg concentrations in porewaters and the fluxes of methylmercury out of incubated cores was not straightforward. Little methylmercury was released from an incubated core collected from the station with moderately reducing sediment and with the highest porewater methylmercury concentration of the study. In contrast, two incubated cores collected from stations with weakly reducing sediment and low methylmercury porewater concentrations released as much methylmercury as most of the stations with reducing and highly reducing sediment. The role of benthic fauna in inhibiting methylmercury release through demethylation or enhancing methylmercury through biological irrigation of the sediments needs further study.

Water column samples collected at four stations 12 times at about monthly intervals between August 2008 and August 2009 helped to develop a better understanding of the seasonal processes affecting mercury in Sinclair Inlet. The variation in water-quality parameters, including mercury, between the Sinclair Inlet stations, or between BNC stations and GSI stations, was similar to variations across Puget Sound. Most notable was the strong seasonal trends in nutrient concentrations from the monthly sampling. Seasonal biological activity also resulted in significant differences between near-bottom and near-surface samples, especially in August 2008 and June 2009. Although several parameters displayed a winter minima, the order of the appearance of that minima is led by the chlorophyll and particulate MHg concentrations and followed by zooplankton mercury concentrations. Concentrations then begin to increase for chlorophyll in January or February, for ^{15}N in March, particulate methylmercury in April, and zooplankton in May. Filtered methylmercury trends were difficult to interpret due to a predominance of samples with results less than the detection limit. This order of the seasonal inflection point in

these parameters is consistent with the hypothesis that the water column production of phytoplankton has a 1 to 2 month delayed stimulatory effect on benthic microbial activity.

Unlike many reactive biogeochemicals, few significant differences of FMHg or particulate methylmercury (PMHg) exist for any of the four seasonal sampling periods. Two instances of extremely high concentrations of FTHg and FMHg provide insight into processes affecting methylmercury in Sinclair Inlet. Vertical water column data suggested that the extremely high concentrations of FTHg and FMHg in near-bottom water from Sinclair Inlet-Port Orchard station in June 2009 was consistent with indications that submarine groundwater discharge pushed reduced porewater into the water column. In contrast, the high concentrations of FTHg and FMHg in near-surface water from the Sinclair Inlet-Inner station in August 2009 were consistent with accumulation of both dissolved and particulate constituents in the surface layer because of a rapid phytoplankton growth (chlorophyll *a* of 150 µg/L) prior to that date. These single outlier observations suggest insight into the system, but provide insufficient evidence for definitive conclusions about those set of conditions.

Acknowledgments

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Appendix 1. Supplementary Figures and Tables

Appendix 1 tables (Microsoft® Excel file) are available for download at https://doi.org/10.3133/sir20185063.
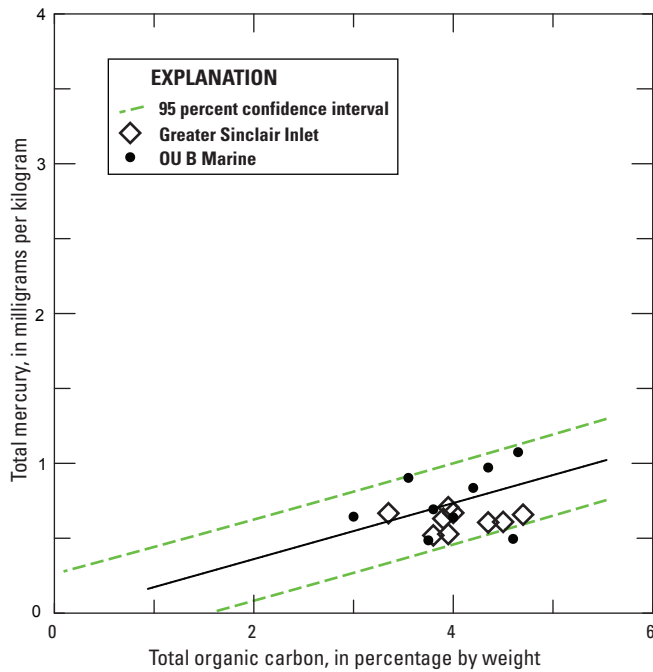

Figure 1-1. Total mercury concentrations and total organic carbon in sediment in and adjacent to Sinclair Inlet, Kitsap County, Washington, 2009. Regression and upper confidence interval is from the 2007 U.S. Navy Long-term Monitoring Program (Paulson and others, 2010).
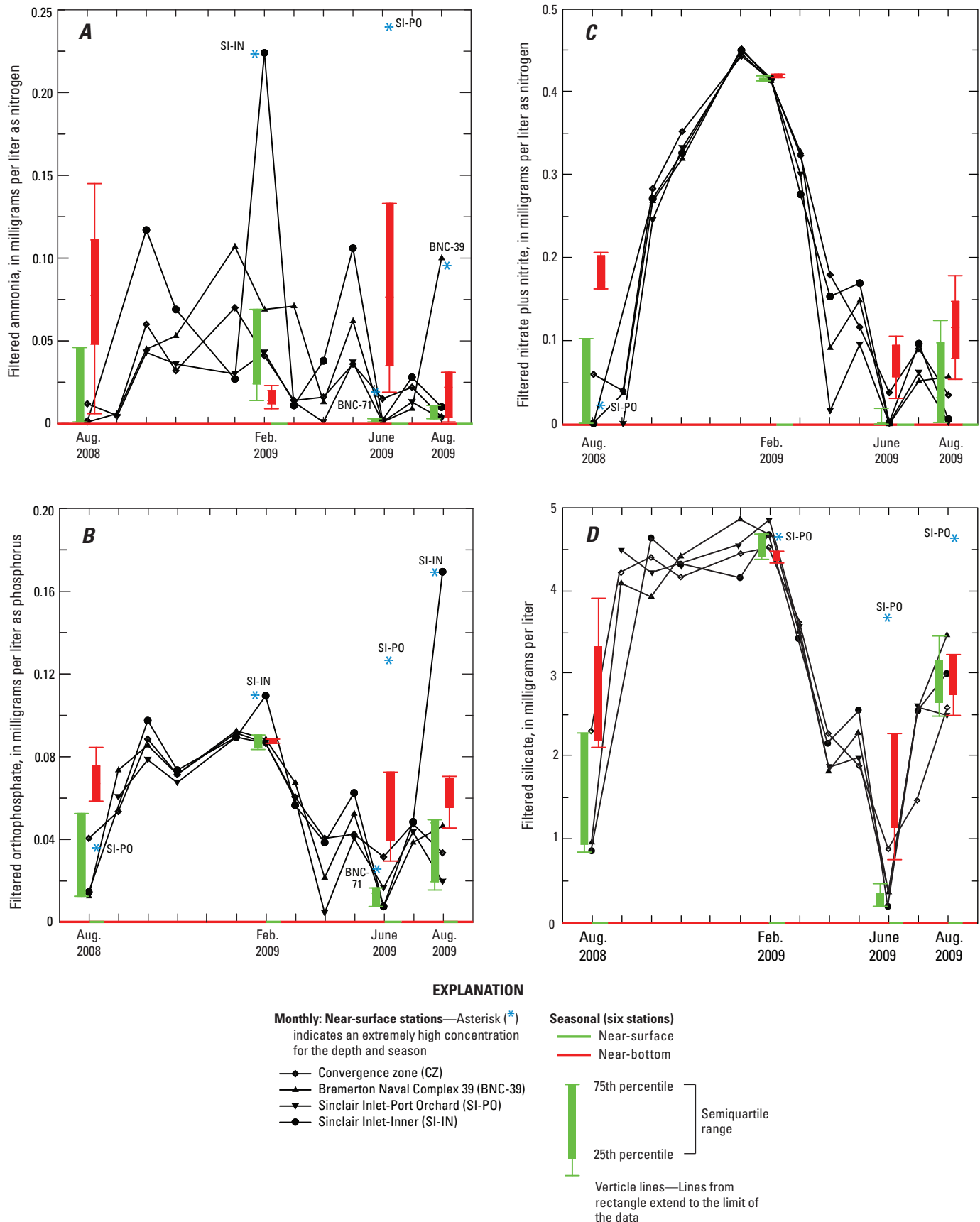
Figure 1-2.   Filtered (*A*) ammonia, (*B*) orthophosphate, (*C*) nitrate plus nitrite, and (*D*) silicate for four monthly near-surface stations and boxplots for six seasonal near-surface and near-bottom stations Sinclair Inlet, Kitsap County, Washington.
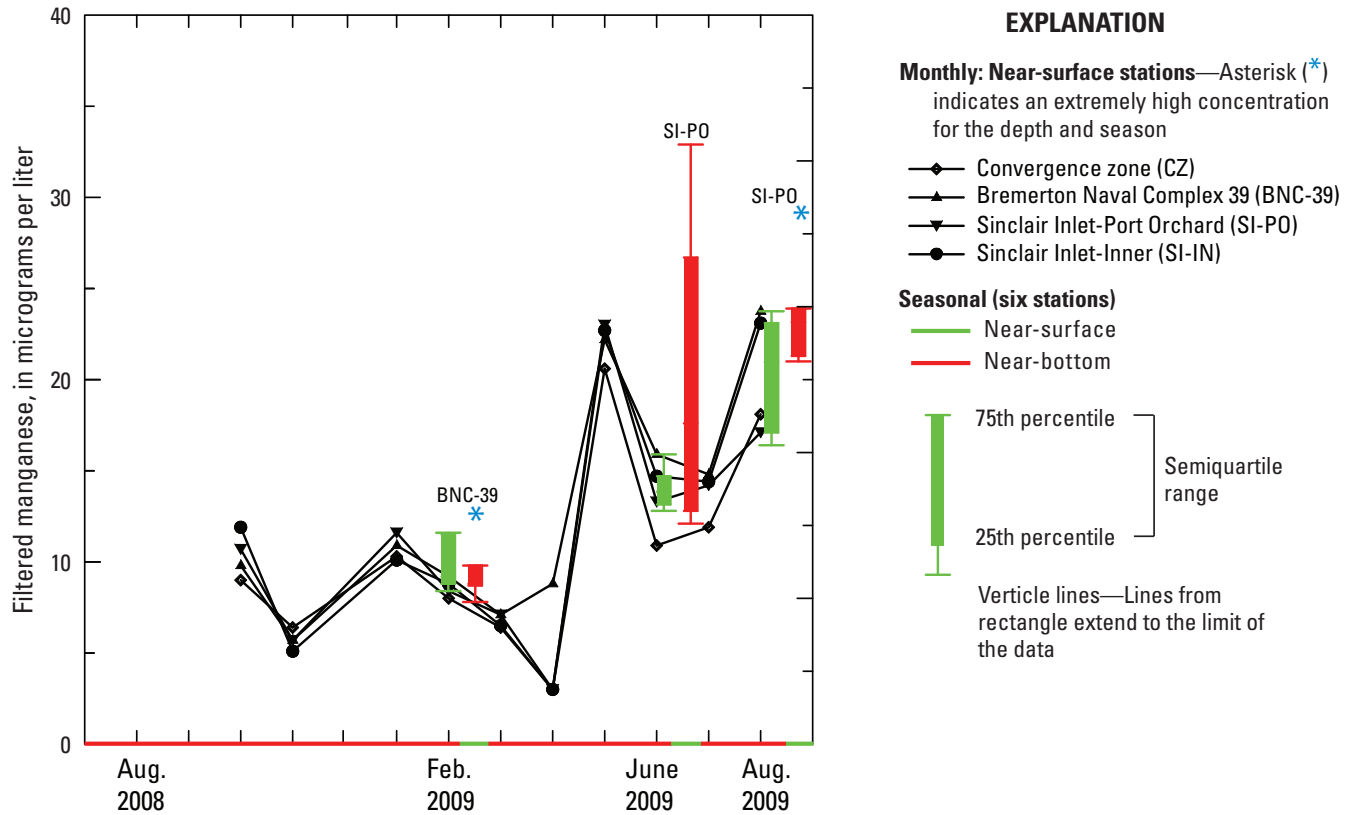
Figure 1-3.   Filtered manganese for four monthly near-surface stations and boxplots for six seasonal near-surface and near-bottom stations, Sinclair Inlet, Kitsap County, Washington.
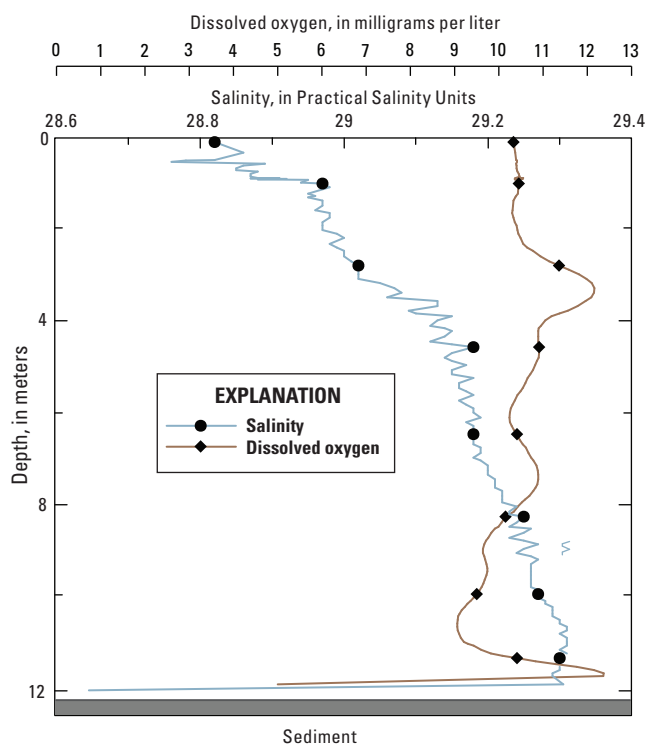
Figure 1-4.   Vertical profile of salinity and dissolved oxygen at Sinclair Inlet station SI-PO, May 9, 2009 (1230), showing evidence of submarine discharge of freshwater near the sediment water interface. Data from Huffman and others (2012, appendix I).

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